Accelerating micro-scale PCR (polymerase chain reactor) for modular lab-on-a-chip system

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ACCELERATING MICRO-SCALE PCR (POLYMERASE CHAIN REACTOR) FOR MODULAR LAB-ON-A-CHIP SYSTEM

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science in Mechanical Engineering
In
The Department of Mechanical Engineering

by
Pin-Chuan Chen
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ABSTRACT

The polymerase chain reaction (PCR) is a powerful technique used to exponentially amplify specific DNA sequences of interest through repetitive temperature cycling. The temperatures used in PCR are typically 90°C - 94°C for denaturation, 50°C - 70°C for renaturation, and 70°C - 75°C for extension. The double-stranded helix of nucleotides, carrying the genetic information, is separated during denaturation, reacts with chemical primers during renaturation, and becomes a complete, replica, double-stranded DNA helix structure during extension. The primary drawback of current commercial benchtop PCR machine is its cycle time due to its large thermal capacitance, and micro PCR is under developing using microfabrication technology; smaller and faster in different types of PCR are the primary goal in this study.

Continuous flow PCR is one of the primary types of micro PCR and it relies on a continuous flow through three temperature zones to achieve rapid thermal cyclings. To understand its biological performance, an experiment was carried out to study its limiting dynamic performance at different flow velocities from 1 mm/s to 15 mm/s and a thermal and fluidic numerical simulation was realized to give ideas of thermal performance and explain the experimental results. A 5.2 s/cycle for 500 bp and a 9.7 s/cycle for 997 bp were accomplished in this experiment. Thermal management was critical in PCR since the biological performance was primarily dependent on precise temperature control. Liquid crystal is a common tool in investigating thermal performance using its optical properties, which will have different color based on its local temperature. A liquid crystal was realized on renaturation zone of CFPCR chip and post image process was used to help interpret the experimental results. A non-uniform temperature distribution was observed due to the low thermal conductivity of substrate, polycarbonate, and non-uniform temperature supply.

Another goal in this research was to develop another type of PCR using different driving mechanism to search other possibilities and compare results with CFPCR. An
electrokineite shuttle PCR was developed and its basic idea was using electrokinetic force to drive DNA fragment through three static temperature zones in a single microchannel. The flow velocities realized in this experiment were 1mm/s to 3mm/s and lower amplification yields were observed. The reasons were unintentional flows such as siphoning flow and hydrodynamic flow, which made the DNA fragment move in a chaotic temperature sequences and result in a lower yield.
CHAPTER 1: INTRODUCTION

1.1 Introduction to PCR

The polymerase chain reaction (PCR) is a powerful technique used to exponentially amplify specific DNA sequences of interest through repetitive temperature cycling [Mullis, et al, 1994]. The temperatures used in PCR are typically 90°C - 94°C for denaturation, 50°C - 70°C for renaturation, and 70°C - 75°C for extension (Figure 1.1). The double-stranded helix of nucleotides, carrying the genetic information, is separated during denaturation, reacts with chemical primers during renaturation, and becomes a complete, replica, double-stranded DNA helix structure during extension. After repeating thermal cycles of the three specific temperatures, the target is copied into billions of molecules. PCR can be applied in medical, academic, or criminal investigations because its output can produce sufficient amounts of the DNA of interest for analysis and detection.

1.2 Three Temperature Zones for PCR

Ideally, DNA cocktail must be cycled through three different temperature zones, 90°C - 94°C for denaturation, 50°C - 70°C for renaturation, and 70°C - 75°C for extension. In practice, preheating and postheating are executed to assure complete denaturation before injection of the DNA cocktail into the micro PCR and complete extension before extraction of the DNA cocktail from the micro PCR [Innis et. al, 1990].

![Figure 1.1: Ideal temperature profile for PCR.](image-url)
DNA templates are separated from double helix structures into two single helix structures during denaturation at 90°C - 94°C. Incomplete denaturation is the most common reason for failure and the accepted solution is preheating before the first thermal cycle [Innis et. al, 1990]. The time needed for the denaturation step during thermal cycles is theoretically less than one second [Wittwer, at el, 1990]. Too high a temperature or too long a dwell time in the denaturation or renaturation zones will lead to a loss of enzyme activity.

The second temperature zone is for renaturation and its required temperature is usually between 50°C and 70°C. At this temperature, the primers attach to the 3’ end of the single helix DNA structure. The temperature and time needed for renaturation depend on the composition of the base, target DNA length, and concentration of the primers [Innis et. al, 1990]. Theoretically the duration less than 1 second is very short, like the denaturation time. [Wittwer, at el, 1990]. Higher renaturation temperatures could minimize non-specific primer annealing, increase the amplification of the specific DNA of interest, and decrease primer-dimer formation, when primers attach to each other instead of gluing to the DNA template.

Extension is the last step in a complete thermal cycle and the temperature varies from 70°C to 75°C. The primers attach to the separated template DNAs from the renaturation step and extend from the 3’ end to the 5’ end to form a complete double helix DNA structure. This step takes much longer than the other two and the dwell time depends on the temperature, concentration, and the length of the target DNA segments. Amplification rates can be expected to be 35 to 100 nucleotides per second based on the reaction environment [Innis et. al, 1990]. Longer extension times for early thermal cycles are helpful for complete amplification because more templates are made in the early thermal cycles, resulting in higher amplification rates and greater specificity [Innis et. al, 1990].

The last step before extracting the DNA cocktail from a micro PCR is postheating, which ensures complete extension. The DNA cocktail is usually kept at the extension
temperature for another five minutes after completion of thermal cycling [Innis et. al, 1990].

1.3 Conventional PCR Device

Figure 1.2: Commercial benchtop PCR thermal cycler [Flexigene, Albany, NY]

Conventional benchtop PCR thermal cyclers (Figure 1.2) are always used in biological and medical research because of their reliability. The primary drawback is the cycle time, which is typically on the order of two hours to complete 20 thermal cycles. This can be longer depending on the DNA segment length and cocktail conditions. The cyclers have large thermal capacitance in the supporting stages for the DNA cocktail. As a result, the heating and cooling of these machines is very slow, motivating much of the research on the PCR. By taking advantage of microfabrication technology, the goal is to design a better PCR device with the same reliability but shorter cycle times. This, in turn, would accelerate the pace of research dependent on thermal cycling technology.

1.4 PCR Requirements

Lab-on-a-chip-based diagnoses may give more reliable results, and consume fewer resources, while accelerating analysis. To overcome a viable alternative to conventional laboratory instrumentation, the power consumption, volume of chemical reagents used, and integration capability of each analytical component must be evaluated for the effect on efficiency, and reliability of the output. This is especially true for PCR, the first step in most DNA-based analyses. The function of the PCR is to provide sufficient samples of interest for diagnosis and investigation by using thermal cycling and primers to amplify the target DNA
segments, even from single DNA molecules. Compared to conventional benchtop PCR instruments, micro PCR devices have shorter reaction times because of lower heat capacitances and higher surface-to-volume ratios. But challenge of controlling temperature still exists especially in cooling from 95°C to 55°C. Different cooling methods, discussed in Chapter 2, have been realized to achieve the renaturation temperature.

Another issue was the choice of material for the PCR device. The goal of this PCR research was to make it reliable and fast medical tool. The material selected would allow easier and cheaper fabrication, be biocompatible, and be disposable to prevent cross-contamination. Polymers are a good choice compared to other popular microfabrication materials like silicon, silicone, and glass. Polymers, like polycarbonate and PMMA, are machinable, optically transparent, have the potential for integration in systems, acceptable thermal and electrical properties, surface modifiable, and less expensive [Soper et al., 2000]. The reason for choosing polycarbonate over PMMA as our CFPCR substrate was its higher glass transition temperature [Mitchell, 2002], the critical temperature at which a polymer loses the properties of a glass, like molecular arrangement, vacancies, induced strain, viscosity, and other factors, and transfers gradually toward the properties of an elastomer.

The LSU continuous flow PCR (CFPCR) is a polymer-based micro device and its challenges are DNA cocktail heat dissipation from 95°C to 55°C and maintaining thermal uniformity across 20 microchannels. Numerical simulation and thermochromic liquid crystals were used to understand and address those questions. Based on ideas developed from CFPCR experimental results and the literature, the shuttle PCR was developed and tested. Thermal management in the shuttle PCR was an important issue and its design and dimensions were realized using numerical simulation. A new critical question encountered in the shuttle PCR was electrokinetic control and hydrodynamic pressure generated from different levels of reservoirs. The experience of other groups and theoretical applied voltage consideration were helpful in minimizing the problems.
1.5 Thesis Outline

This research focused on investigating the performance of the LSU continuous flow PCR (CFPCR) [Hashimoto et al., 2004] and developing a next generation PCR, the shuttle PCR.

Chapter 2 is a review of PCR methods an instrumentation, current PCR research, and the requirements for better performance. Validation of the LSU continuous flow PCR is presented in Chapter 3, with results of numerical thermal and fluidic simulations of limiting performance at different flow rates, improvement in microfabrication and assembly, and measurement of the temperature distribution in the renaturation zone by using thermochromic liquid crystals. Chapter 4 introduces the shuttle PCR including the basic concepts, steady-state and transient thermal analyses, thermal and molecular diffusion considerations, microfabrication and assembly, and experiment evaluation of the performance. A summary of the achievements of the current work, conclusions, and proposed future work are included in Chapter 5. Additionally the Appendices list the comparison among different PCRs.
CHAPTER 2: BACKGROUND

2.1 Introduction

The function and requirements for PCR were introduced in Chapter 1. Chapter 2 introduces different PCR devices reported by other research groups, alternative driven mechanisms for PCR, and motivations behind this study.

2.2 Summary of Micro PCR Types

Several groups have investigated factors that would lead to faster and more reliable PCR devices. PCR devices can be cataloged using substrate materials, drive mechanisms, and heating/cooling mechanisms. In Appendix A1, some PCR devices are listed for comparison with their reagent volumes, reaction time, substrate materials, and cycle numbers. Generally speaking, micro PCR devices can be separated into two major types: micro chamber PCR and micro continuous flow PCR (CFPCR). There have also been some creative PCR devices reported that do not fit under these classifications. Micro chamber PCR devices are miniaturized versions of commercial benchtop PCR instruments. The DNA cocktail remains in a single chamber while heating and cooling elements are repeatedly thermally cycled. One advantage of the micro chamber PCR is that the chemical reagent volume is constrained only by the microfabricated chamber size and reaction requirements. However, the chamber containing the DNA cocktail must also be heated and cooled, potentially slowing down the overall thermal cycling rate due to the thermal capacitance of the device. Continuous flow PCR (CFPCR) takes advantage of the low thermal capacitance and high thermal conductivity of the DNA cocktail in the microchannels, which allows it to quickly reach thermal equilibrium with the channel walls in each distinct temperature zone upon entering a zone. This accelerates the PCR process, because the CFPCR does not expend as much power and time cycling the device temperature. However, the volume of chemical reagents needed for CFPCR on the micro scale is much greater than is typically required for micro chamber PCR because the entire channel must be filled.
2.2.1 Performance of Commercial PCR Devices

PCR is a developed technology for chemical and biological analysis, but it still has room for improvement relative to the current block thermal cyclers. The PCR cocktail is held in a multi-well rack in a metal block driving the thermal cycling. Although the metal rack can be reliably utilized for PCR but the time needed is typically few hours. An efficient lab-on-a-chip is relied on each quick and accurate micro device so few-hour operation for a basic analysis step, amplification of interested DNA, is hard to accept and that is major motivation behind PCR development.

Currently a company, Cepheid (Sunnyvale, CA), is selling an integrated system, GeneXpert, which has functions including sample preparation, DNA amplification and detection. The time needed for this system to analyze a sample is less than 30 minutes. It can handle every step from raw sample to analysis of results automatically. The raw sample is injected into a cartridge which plugs into the main system, which has a size similar to a personal computer, for operation and analysis while analysis results will be exported via computer later. The demerit of this system is its size. Miniaturizing this kind of integrated system as a lab-on-a-chip is a goal for improving Bio-MEMS technology.

2.2.2 Micro Chamber PCR

Several micro chamber PCR devices have been reported. Groups used different substrate materials, microfabrication technology, and heating/cooling methods to achieve faster amplification performance.

A silicon substrate was micromachined to make a 2 µl vessel using anisotropic wet etching [Daniel et al, 1998]. The heater and sensor were realized using thin film platinum resistors connected to a computer running LabView software. The main micro chamber was suspended by four beams and partially isolated from the substrate. The merit of this design was to decrease the thermal capacitance during heating and increase the cooling rate; the natural convection from the bottom contributed because the heaters were fabricated on the
four beams only. The target PCR fragment was 260 bp and the total cycles were 30 cycles with 94°C for 5 seconds, 55°C for 7 seconds, and 72°C for 5 seconds. Preheating or postheating was realized for 1 minute.

Giordano [Giordano et al, 2001] made a micro chamber PCR device with infrared-mediated temperature control on a polyimide substrate. A 50 W tungsten lamp was used as a non-contact heat source and was focused on the micro chamber through a convex lens. A small 0.002” diameter T-type thermocouple was arranged in the chamber to measure the temperature directly. The reagent volume was 1.7 µl and 15 cycles were completed in 240 seconds (16 seconds/cycle).

Polydimethylsiloxane (PDMS) was used as a PCR device substrate with 2 µl volume [Shin et al, 2003]. The PDMS was porous, which caused bubble formation, sample evaporation, and protein adsorption. Parylene was coated on the surface of the chamber to reduce these problems. Parylene was also used to make the treated surface more hydrophobic and resist DNA absorption; this coating step was especially critical for PDMS because the plasma surface treatment necessary for PDMS bonding would make the surface more hydrophilic and increase the possibility of failed PCR amplification. The cycle time used was 3 minutes for preheating, 15 seconds for denaturation, 15 seconds for renaturation, 30 seconds for extension, and 10 minutes for postheating; total cycles were 30 cycles. Double-sided heating was realized using alumina blocks to sandwich the PDMS chip because of the low thermal conductivity of the PDMS and forced cooling was done using an electric fan from the side of the PDMS chip. The heating and cooling rates could reach at 2°C/s and 1.2°C/s, respectively.

A micro-chamber array for PCR with different reactor volumes ranging from 1.3 pl to 32 µl was made on a silicon substrate to exploit its reduced PCR cocktail demand [Nagai et al, 2001]. Reactor volumes larger than 86 pL gave successful amplification with preheating at 94°C for 1 minute, 40 cycles consisting denaturation for 1 minute, renaturation for 1 minute,
extension for 1 minute. For reactor volumes smaller than 86 pL, no product was detected after the amplification process.

2.2.3 Continuous Flow Micro-PCRs

A second kind of micro-PCR is the continuous flow PCR (CFPCR). It relies on a continuous flow of reagent through three thermostatic zones: denaturation, renaturation and extension. At the microscale, it was initially assumed that thermal balance was achieved quickly, which was validated by simulation.

For the first reported CFPCR device, Kopp [Kopp et al, 1998] used glass as a substrate with an independent copper block to support each temperature zone. The high thermal conductivity of the copper block produced a uniform temperature distribution in each temperature zone. The microchannel was etched on a glass and its length was 2.2m with 40 micros deep and 90 micros wide. Flow rates ranging from 5.8 nl/s to 72.9 nl/s were used to amplify a 176 base pair fragment DNA cocktail. PCR results were obtained from 1.5 min to 18.7 min to for 20 cycles with 10 µl of reagents.

At LSU, a CFPCR was fabricated using LIGA and UV-LIGA to pattern mold inserts (Figure 2.1) that were used to hot emboss devices in polycarbonate. The low thermal conductivities of air and polycarbonate were used to make three distinct temperature zones [Mitchell et al., 2002]. Commercial thin-firm heaters were used as heat sources for each zone and cooling was by natural convection, with PID control of the temperature in each zone. Finite element simulation (ANSYS, vers. 8.1, Canonsburg, PA) was used to investigate the design parameters and thermal performance for a single, 2-D microchannel. In the simulations, these assumptions were made: isotropic thermal and fluidic properties for water, polycarbonate and air; laminar, incompressible, Poiseuille flow because of low Reynolds number (Re<<1); and two-dimensional heat transfer (simulation parameters are listed in Table 3.1). The actual device had 20 spiral cycles with a nominal ratio of in each zone of 1:1:4 (denaturation: renaturation: extension).
Low temperature co-fired ceramics (LTCC) were used as a substrate material for CFPCR by a group at Motorola [Sadler et al, 2003]. Air gaps were used between temperature zones to isolate the zones for isolation. A larger microchannel was incorporated in the extension zone to enable longer residence times than in the other two zones since extension needed more time, depending on the length of DNA segment, than denaturation and renaturation. A thermal numerical simulation was applied for development and optimization of this device; the device and air gap dimensions were decided after deliberate consideration to realize the isolation of each temperature zone. The DNA cocktail, 24 µl, was pumped into the microchannel and recirculated through the device using an external pump. Forty cycles, consisting of 10 seconds for denaturation, 20 seconds for renaturation, and 30 seconds for extension, were used in the experiments. A 209 bp fragment was amplified successfully on this ceramic micro-chamber device.

Obeid made their CFPCR on glass combined with laser-induced fluorescence (LIF) as a detection system [Obeid et al, 2003]. Different inlet positions for choosing from 20 to 40 cycles in increments of 5 were incorporated in the device. A parametric study of the impact of different molecular concentrations and different PCR cycle numbers on amplification results was carried out. A DNA fragment, 230bp, was pumped into this micro device and then stained with fluorescent dye for analysis. Amplification was carried out for 30 cycles with a flow rate 21nl/s to study the input DNA molecules. Using LIF gave a 16 fold improvement in detection compared to convectional agarose gel electrophoresis and enabled detection of
DNA product corresponding to 3900 DNA template molecules. The PCR cocktail, 10µl corresponding to $5 \times 10^6$ DNA molecules at 21 nl/s, was pumped through different numbers of thermal cycles to investigate the effect. The product increased exponentially and the largest increase happened between 30-40 cycles.

### 2.2.4 Other Micro-PCR Configurations

Some PCR development could not be easily cataloged into the previous sections but offered an alternative developments in PCR technology.

Arrays of poly(dimethylsiloxane) (PDMS) micro pumps and micro valves were developed at Caltech. [Quake et al, 2001]. Pneumatic actuation deflected the PDMS membranes that would block or open underlying channels. One of the applications tested was matrix PCR. Thermal cycling was applied to the whole chip with a protocol of 50°C for 2 minutes, 96°C for 1 minute, 30 cycles of 20 seconds at 96°C and 40 seconds at 60°C; the total time including ramping and cooling time was 1 hour. The advantage of this device was that there were 400 distinct PCR devices on the same matrix chip that could be loaded with just 40 pipetting steps. Up to 41 chemical reagents could be injected simultaneously and distributed to each reaction vessel using the micro valves. After injection, the peristaltic pumps, using deflecting PDMS micro pumps to control flow direction underneath the chip, were operated for uniform mixing. Amplification of a 294 bp DNA segment was performed.

Nakano reported a water-in-oil emulsion that included multiple droplets of reaction mixture acting as micro reactors [Nakano et al, 2003]. The first PCR was done with the DNA cocktail encapsulated in silicone oil. This step was used to preamplify the DNA template for use in a subsequent PCR step especially for very low molecular concentration. The first PCR also minimized undesired amplification, primer-dimers, and DNA cocktail adsorption especially in the initial few cycles because they were the templates of later cycles. In the first water/oil emulsion PCR, 50µl PCR cocktail mixed with 950µl silicone oil was stirred to make
numerous PCR droplets for 13 thermal cycles which applied 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute. Later the amplified DNA from the first PCR was centrifuged and further amplified in a conventional PCR instrument. The second step was also had 13 cycles with 95°C for 30 seconds, 66°C for 30 seconds, and 72°C for 1 minute. The agarose gel analysis listed four different concentrations of DNA template, 10 molecules, 1 molecule, 0.1 molecule, and 0 molecule for comparison. This method was successfully used to amplify DNA from a single molecule even 0.1 molecule.

A concept for a bi-directional peristaltic pump to act as the driving source to transport DNA cocktail droplets between three different isothermal chambers was reported [Bu et al, 2003]. The sequence of the three temperature zones arranged along a single channel was 95°C, 72°C and 55°C. A theoretical evaluation of the deflection of the peristaltic micro pump and numerical simulations were used to assess the temperature distribution in the micro chambers. It was proposed to deposit Pt as heaters and sensors embossed in the chip and use optical fibre to track the chemical reagent positions. The DNA droplet was designed to be 1 µl injected into the reservoir. Deflection of the pump membrane was analyzed using analytical and FEM model.

Another group [Wang et al, 2004] also developed a single straight microchannel layout with a sequence of 93°C, 72°C, 50°C zones, respectively. Numerical simulation and the quasi multi-systems lumped heat capacity method were used to analyze the thermal performance of the device. A prototype was built on a silicon substrate and grooves were etched around the microchannel to achieve thermal isolation. A set of 35 thermal cycles were used for the PCR process with 2 minutes for preheating at 93°C, 4 seconds at 93°C of denaturation, 4 seconds at 50°C of annealing, and 4 seconds at 72°C of extension, and 4 minutes at 72°C of postheating.

2.2.5 Micro-PCR Summary

PCR is a well-developed technology in most chemical and biological laboratories
according performed with reliable commercial thermal cyclers. There still is need for improved speed and portability. Micro chamber PCR, continuous flow PCR, and other different types of PCR were described in the previous sections; each specific layout has its own merits but also disadvantages. An important point in designing the current PCR is how to make this device easier to integrate with other devices for a lab-on-a-chip.

2.3 Drive Mechanism Comparison

The drive mechanism is a critical issue in microfluidic devices because driving and manipulating technologies are relative to the performance of a device and integration with other functional devices.

Pressure driven flow is the most common drive mechanism in microfluidic PCR except for micro chamber type PCR. The benefits of pressure drive include ease of operation and easy to work and reliability, but the demerits are the back pressure generated during the flow through the microchannel; the smaller the microchannel, the larger the pressure. Bonding and connection on pressure-driven chips must survive under this back pressure. The LSU CFPCR used a syringe pump as a source to pump the PCR cocktail through the 20 thermal cycles, 1.78 meter long microchannel. The pressure gradient needed for 2mm/s flow in the 50 micros wide and 150 micros deep microchannel was $1.2152 \times 10^4 \text{Pa/m}$ [Mitchell et al., 2002].

Another drive mechanism was electrokinetics. On the micro scale, surface forces dominate volume force due to the very high surface-to-volume ratio. Electrokinetic phenomenon utilize the electric double layer, generated by an ionic fluid contacting charged microchannel walls, to pull the bulk fluid move. Electrokinetic phenomenon are already realized in many applications like manipulating fluids in the microchannel or capillary electrophoresis. A problem with this mechanism is diffusion; molecular diffusion, thermal diffusion, and hydrodynamic diffusion generated from unbalanced levels of reservoirs can contribute and there are no efficient methods of controlling diffusion to date. Chen developed
an electrikinetically-driven circular PCR to amplify a 500 bp segment of DNA [Chen, et al. 2005]. A dynamic coating, hexadimethrine bromide (Polybrene, PB), was applied to the microchannel to reverse the electroosmotic flow direction, minimizing the diffusion generated from the opposing electroosmotic flow. This PCR was similar to CFPCR, with the DNA cocktail following a square microchannel which had one side for denaturation at 94°C, one side for annealing at 55°C, and two sides for extension at 72°C repeatedly. The total length of this device was 7.6 cm and the time needed for a cycle was 40.16 seconds based on 300V/cm. An interesting study was carried out in this paper on minimizing Joule heating. Removing KCl, a common component of PCR cocktails, made the current much smaller but had no effect on PCR products. Five different concentrations of KCl mixed with PCR cocktail were used for comparison.

A pneumatic system was used by Chiou as a driver to control the position of a DNA cocktail droplet in a PTFE capillary with three different temperature zones [Chiou et. al, 2001]. This capillary was suspended on heater blocks and connected to computer-controlled valves. A droplet, with 1µl PCR cocktail, was injected into the capillary and amplification of a 500 base-pair DNA was obtained in 46 seconds per cycle. Photodiodes were used for detection of the droplet position in the capillary. The dwell times within each temperature zone were 2 seconds, 2 seconds, and 30 seconds for denaturation, renaturation, and extension, respectively. Different temperature ranges for each zone was studied, denaturation from 88°C to 98°C, annealing from 54°C to 72°C, and extension from 70°C to 80°C. Denaturation at 98°C, annealing at 56°C, and extension at 70°C gave the best results according to the yield concentration.

2.4 Conclusions on Previous Related Work

PCR has developed well in benchtop technology but a goal of minimizing its size is ongoing using micro fabrication technology. Micro chamber PCR and CFPCR are the two most common configurations but other kinds of PCR also obtain some results and play an
important role in the evolution of micro PCR. Different drive mechanisms are realized for obtaining good amplification results as well as minimizing device size. Thermal management is an important issue since temperature will affect Bio-MEMS device’s performance.
CHAPTER 3: LSU CONTINUOUS FLOW PCR AND ITS PERFORMANCE

The performance of the LSU CFPCR was assessed at different flow rates using a combination of thermal and fluidic numerical simulations, and experiments. Thermal distribution on the CFPCR chip was measured using thermochromic liquid crystals. The CFPCR devices were molded using a mold insert fabricated via the LIGA microfabrication process described in previous work [Mitchell et al., 2002]. A critical problem in CFPCR is how to lower the temperature of the PCR reagents from 95°C in the denaturation zone to 55°C in the hybridization zone, when only natural convection is utilized for CFPCR device.

3.1 Thermal and fluidic simulation of CFPCR

3.1.1 Numerical model configuration and description

ANSYS/CFD-FLOTRAN (vers. 8.1, ANSYS, Inc., Canonsburg, PA) was used to help understand the temperature distribution along the single microchannel while varying the flow velocity. The actual CFPCR had 20 spiral cycles and the finite element model considered only one straight microchannel (see Figure 3.1). Since the width of the microchannel (50 µm) was much smaller than the width of heating area, adiabatic thermal boundary conditions were applied on each side surface while the top and bottom were exposed to natural convection with a coefficient of 15 W/m², an average convection coefficient at room temperature [Incropera et al., 1996].

The inlet and outlet were also assumed to be adiabatic. Figure 3.1 (b) shows a cross-section view of the single microchannel model with the boundary conditions. The commercial thin firm heaters were located in the upper and lower airpockets to apply uniform heat flux to the sandwiched microchannel. The ambient temperature was assumed to be 24°C. Radiation was considered with conduction inside airpockets [Mitchell et al., 2002], but convection was negligible. The isotropic thermal and the fluid properties of water were assumed as properties of the DNA cocktail. The fluid was assumed to be a fully developed laminar, Poiseuille flow. Table 3.1 lists the thermal and fluid properties of air, polycarbonate,
and water as a function of temperature.

![Diagram](image)

Figure 3.1(a)(b): Configuration of single microchannel model and its thermal boundary conditions.

Since the upper part and lower part of CFPCR were symmetrical, only the upper part was included in the simulation model. Figure 3.2 is the cross-section of the symmetric model used in the ANSYS (vers. 8.1, ANSYS, Inc., Canonsburg, PA). The center block of the bottom layer was the microchannel and the other two neighboring blocks were polycarbonate. In the actual CFPCR device, the distance between each microchannel was a 250 µm so the width of each polycarbonate block in the bottom layer was 125 µm.
Table 3.1: Thermal and fluidic properties of air, fluid and polycarbonate.

<table>
<thead>
<tr>
<th>Device Dimension</th>
<th>Model Dimension</th>
<th>Micro-channel dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>6 cm</td>
<td>6 cm</td>
</tr>
<tr>
<td>Width</td>
<td>150 µm</td>
<td>50 µm</td>
</tr>
<tr>
<td>Height</td>
<td>1325 µm</td>
<td>150 µm</td>
</tr>
</tbody>
</table>

Polycarbonate (Goodfellow, UK)
- Thermal Conductivity: 0.2 W/m-K
- Specific Heat: 1200 J/kg-K
- Density: 1200 kg/m³

Fluid Properties (Water at 74°C)
- Thermal Conductivity: 0.668 W/m-K
- Specific Heat: 4191 J/kg-K
- Density: 1024 kg/m³
- Viscosity: $3.89 \times 10^{-4}$ N*s/m²

Air Properties
- 68°C
  - Conductivity (W/m-K): 0.0293
  - Specific Heat (J/kg-K): 1008
  - Density (kg/m³): 1.017
- 72°C
  - Conductivity (W/m-K): 0.0296
  - Specific Heat (J/kg-K): 1008
  - Density (kg/m³): 1.0074
- 95°C
  - Conductivity (W/m-K): 0.0313
  - Specific Heat (J/kg-K): 1012
  - Density (kg/m³): 0.95

Figure 3.2: Cross-sectional configuration of the symmetric CFPCR model.
The polycarbonate just above the microchannel was 250 µm thick coverslip. The airpockets were made by micro milling 600 µm deep cavities on a 1 mm thick polycarbonate stockship. The total length of the channel in the simulation model was 6 cm and the ratios of denaturation, renaturation and extension were 1:1:4. The total height of the model was 1.325 mm. A no-slip boundary condition was applied to the top and two side walls inside the microchannel. The bottom surface, the symmetry plane of the microchannel, was assumed to be a free boundary. Pressure was applied on the inlet to drive the DNA cocktail through the microchannel with zero pressure applied at the outlet.

3.1.2 Convergence Investigation for ANSYS-FLOTRAN Simulation Model

The mesh elements for the ANSYS-FLOTRAN (vers. 8.1, ANSYS, Inc., Canonsburg, PA) simulation were 3-D brick elements for the microchannel and fluid, and 3-D tetrahedral elements for airpockets and polycarbonate followed from ANSYS instruction example. Grid sensitivity was checked by using different size elements to ensure that results were independent of element sizes. Table 3.2 lists the numbers of different mesh elements based on choosing different sizes of either brick or tetrahedral elements. The brick element was fixed (6 × 6 × 100) while changing the tetrahedral element size in the airpockets and polycarbonate as the tetrahedral element was the same (smart 5) during changing brick element size of microchannel and fluid.

Figure 3.3: The steady-state temperature distribution along microchannel for three different tetrahedral element sizes for the airpockets and polycarbonate.
Table 3.2: Different mesh sizes and element numbers for checking convergence.

<table>
<thead>
<tr>
<th>ANSYS-FLOTRAN numerical simulation model</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Airpockets / Polycarbonate</td>
<td>Microchannel / Fluid</td>
</tr>
<tr>
<td>6 × 6 × 100 (fixed brick element)</td>
<td>Smart 5 (fixed tetrahedral element)</td>
</tr>
<tr>
<td>Smart 1</td>
<td>23163</td>
</tr>
<tr>
<td>Smart 5</td>
<td>22282</td>
</tr>
<tr>
<td>Smart 9</td>
<td>17549</td>
</tr>
</tbody>
</table>

Figure 3.3 and 3.4 show the steady-state temperature distributions along the microchannel for simulation using different size meshing elements for a 2mm/s flow. In Figure 3.3 the tetrahedral element size used for the airpockets and polycarbonate was changed, while in Figure 3.4 changed the size of the brick elements modeling the microchannel were changed.

Figure 3.4: The temperature distribution along the microchannel for different element sizes in the microchannel.
Varying the brick element size in the microchannel had a more significant effect on the resulting temperature distribution. From Table 3.2, the number of $7 \times 7 \times 120$ elements was 1.23 times greater than for $6 \times 6 \times 100$. There were 1.17 times more $6 \times 6 \times 100$ elements than $3 \times 3 \times 150$. The ratios were similar while the temperature difference at 95°C, 4.92°C, between $(7 \times 7 \times 120)$ and $(6 \times 6 \times 100)$ was much smaller than that, 37.88°C, between $(6 \times 6 \times 100)$ and $(3 \times 3 \times 100)$. The large temperature difference between $(6 \times 6 \times 100)$ and $(3 \times 3 \times 100)$ might be due to less elements. This led to the conclusion that the simulation result was converging.

### 3.1.3 The Comparison between Innermost and Outermost Microchannels

In the CFPCR device, the inner microchannel is shorter than the outer microchannel because of the decrease in radius. The innermost microchannel was the one which exactly satisfied the 1:1:4 time proportions.

The length of the denaturation and renaturation zones was 1 cm and the extension zone length was 4 cm. As the DNA cocktail moved to the outer microchannels, the transition and residence times increased compared to the innermost microchannel. Table 3.4 is a comparison between the innermost and outermost microchannels.

Table 3.3: The temperature difference between different brick size elements and the position was 6 mm measured from the inlet.

<table>
<thead>
<tr>
<th>Element size</th>
<th>$3 \times 3 \times 100$</th>
<th>$6 \times 6 \times 100$</th>
<th>$7 \times 7 \times 120$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max temperature (°C )</td>
<td>137.83</td>
<td>99.95</td>
<td>95.03</td>
</tr>
<tr>
<td>Temperature Difference (°C ) between each meshing model at 95 °C</td>
<td>37.88</td>
<td>4.92</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.5: Steady-state temperature distribution comparison between the innermost and outermost microchannels.

The total length increased by 59.7% of the innermost microchannel while the length ratios for the temperature zones decreased from 1:1:4 on the innermost microchannel to 1:1:3 on the outermost microchannel. Figure 3.5 shows the steady-state temperature distributions along the innermost and outermost microchannels. The DNA cocktail traveled a larger distance and spent more time in each temperature zone as it moved to outer spirals.

3.1.4 Single Microchannel Thermal and Fluidic Simulation without Substrate

In PCR the denaturation and renaturation reactions are theoretically completed in less than 1 second and the extension reaction, takes 25-100 bp/second [Innis et. al, 1990]. Finite element analysis was used to understand variation in the steady-state temperature distribution along the microchannel based as the flow rate was increased from 0 mm/s to 20 mm/s.

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Total Time (s)</th>
<th>Ratio of each temp. zone</th>
<th>Total residence time percentage</th>
<th>Total transition time percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner channel</td>
<td>60</td>
<td>30</td>
<td>1:1:4</td>
<td>90</td>
</tr>
<tr>
<td>Outer channel</td>
<td>95.82</td>
<td>47.91</td>
<td>1:1:3</td>
<td>91.99</td>
</tr>
</tbody>
</table>
In principle, increasing the flow rate would speed up the PCR process, with the potential penalty that the available time for heat transfer to the DNA cocktail was reduced. A theoretical model, derived by Dryden in 1959, was used to estimate the theoretical maximum velocity in the rectangular microchannel [Mitchell, 2002]. Figure 3.6 shows the cross-section of the CFPCR microchannel, the width (2a) of 50 µm and the height (2b) of 150 µm used in the model.

For the rectangular section where \(-a \leq y \leq a\) and \(-b \leq z \leq b\), the velocity profile was given by:

\[
u(y,z) = \frac{16a^2}{\mu \pi^3} \left( -\frac{dp}{dx} \right) \sum_{i=1,3,5, \ldots}^{\infty} (-1)^{(i-1)/2} \left[ 1 - \frac{\cosh(i\pi y/2a)}{\cosh(i\pi b/2a)} \right] \times \frac{\cos((i\pi y/2a))}{i^3}
\]

(3.1)

where \(dp/dx\) is the pressure gradient given in terms of the mean velocity, which is:

\[
\left[ \frac{dp}{dx} \right]^{-1} = \frac{a^2}{3} \left[ 1 - \frac{1}{u_{\text{mean}}} \sum_{i=1,3,5, \ldots}^{\infty} \frac{192a}{\pi^5 b} \tanh \left( \frac{i\pi b}{2a} \right) i^3 \right]
\]

(3.2)

By using these equations, the maximum velocity was calculated for each flow rate. In Table 3.5, the maximum mean velocity was the largest velocity on the parabolic pressure-driven flow profile. The time for a single cycle was calculated for the 6 cm long microchannel, the length of the innermost microchannel in the CFPCR device, using the mean velocities. The time to complete 20 cycles was calculated for the total length of the
actual CFPCR microchannel, 1.78 m.

The pressure difference was increased between the inlet and outlet to increase the velocity of the DNA cocktail in the simulated single microchannel. Figure 3.7 shows the results of the finite element thermal and fluidic simulations. The vertical dashed lines show each target temperature range from the inlet. The horizontal bold black lines on the figure define a band within ± 2 degrees of the target temperatures to assist comparison of the system thermal performance and predict the output. The target temperature for denaturation was 95 °C, renaturation was 68 °C, and extension was 72 °C. The peak range (from 95 °C to 68 °C) was shortened and the peak was shifted as the velocity increased.

Table 3.5: The maximum mean velocity and time for a single microchannel and the total CFPCR channel length.

<table>
<thead>
<tr>
<th>Mean Velocity (mm/s)</th>
<th>Max Velocity (mm/s)</th>
<th>Single Cycle Time (second)</th>
<th>20 Cycles Time (second)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.7</td>
<td>30</td>
<td>890</td>
</tr>
<tr>
<td>3</td>
<td>5.6</td>
<td>20</td>
<td>593</td>
</tr>
<tr>
<td>4</td>
<td>7.5</td>
<td>15</td>
<td>445</td>
</tr>
<tr>
<td>5</td>
<td>9.4</td>
<td>12</td>
<td>345</td>
</tr>
<tr>
<td>6</td>
<td>11.2</td>
<td>10</td>
<td>296</td>
</tr>
<tr>
<td>10</td>
<td>18.7</td>
<td>6</td>
<td>178</td>
</tr>
<tr>
<td>15</td>
<td>28.1</td>
<td>4</td>
<td>118</td>
</tr>
<tr>
<td>20</td>
<td>37.4</td>
<td>3</td>
<td>89</td>
</tr>
</tbody>
</table>
Figure 3.7: Temperature distribution along the microchannel for different mean velocities.

Between 2 and 6 mm/s, the dwell time, the time of DNA cocktail staying in target temperature bands, in each target temperature zone was reduced while the transition time, the time of DNA cocktail changing its temperature, between zones was increased. When the velocity was above 10 mm/s, the fluid did not reach 95°C, the target denaturation temperature; at 20 mm/s the peak temperature was 5°C below 95°C at the end of the denaturation zone. Also when the velocities were above 10 mm/s, the target extension temperature, 72°C, was not reached until the fluid had traveled nearly one quarter of the way into the extension zone. The renaturation zone did not have any heating element and the lowest temperature was 68°C from the numerical simulation; so the lower renaturation temperature needed an active cooling system instead of natural convection.

On the ideal temperature curve (Figure 1.1), the transition time was zero. In the practice, the DNA cocktail temperature needed some time to equilibrate with each temperature zones. Table 3.6 predicts the times for transition and dwell based on the FE simulation. When the flow rate was increased, the residence time in the target zones (95°C, 72°C and 68°C) decreased, while the transition times between zones (72°C-95°C, 95°C-68°C and 68°C -72°C) increased. The times for the 72°C -95°C transition and 95°C at 15 mm/s and
20mm/s were zero because the temperature did not reach the 93°C lower tolerance bound for 95°C. The 1.161 seconds for the 15mm/s case and 0.985 seconds for the 20mm/s case were counted as part of the transition time from 95-68 °C. The extension time was the largest because of the microchannel layout and chemical reaction requirements.

Table 3.6: Residence and transition times as a function of the fluid velocity.

<table>
<thead>
<tr>
<th>Mean Velocity (mm/s)</th>
<th>72-95 °C (s)</th>
<th>95 °C (s)</th>
<th>95-68 °C (s)</th>
<th>68 °C (s)</th>
<th>72 °C (s)</th>
<th>Single Cycle Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.154</td>
<td>2.73</td>
<td>1.849</td>
<td>4.578</td>
<td>19.689</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>0.924</td>
<td>1.721</td>
<td>1.325</td>
<td>2.973</td>
<td>13.057</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>0.801</td>
<td>1.221</td>
<td>1.07</td>
<td>2.168</td>
<td>9.74</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>0.727</td>
<td>0.927</td>
<td>0.912</td>
<td>1.69</td>
<td>7.744</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>0.677</td>
<td>0.726</td>
<td>0.805</td>
<td>1.374</td>
<td>6.418</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>0.584</td>
<td>0.283</td>
<td>0.636</td>
<td>0.741</td>
<td>3.756</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>1.161</td>
<td>0.416</td>
<td>2.423</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0.985</td>
<td>0.251</td>
<td>1.764</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3.7: Residence and transition time percentage as a function of the fluid velocity.

<table>
<thead>
<tr>
<th>Mean Velocity mm/sec</th>
<th>72-95</th>
<th>95</th>
<th>95-68</th>
<th>68</th>
<th>72</th>
<th>Total Dwell percent cycle</th>
<th>Total Transition percent cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.84</td>
<td>9.1</td>
<td>6.16</td>
<td>15.2</td>
<td>65.6</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>4.62</td>
<td>8.6</td>
<td>6.625</td>
<td>14.8</td>
<td>65.2</td>
<td>88.6</td>
<td>11.245</td>
</tr>
<tr>
<td>4</td>
<td>5.34</td>
<td>8.14</td>
<td>7.13</td>
<td>14.4</td>
<td>64.9</td>
<td>87.44</td>
<td>12.47</td>
</tr>
<tr>
<td>5</td>
<td>6.05</td>
<td>7.7</td>
<td>7.6</td>
<td>14</td>
<td>64.5</td>
<td>86.2</td>
<td>13.65</td>
</tr>
<tr>
<td>6</td>
<td>6.77</td>
<td>7.26</td>
<td>8.05</td>
<td>13.74</td>
<td>64.18</td>
<td>85.18</td>
<td>14.82</td>
</tr>
<tr>
<td>10</td>
<td>9.73</td>
<td>4.71</td>
<td>10.6</td>
<td>12.35</td>
<td>62.6</td>
<td>79.66</td>
<td>20.33</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>29</td>
<td>10.4</td>
<td>60.5</td>
<td>70.9</td>
<td>29</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>32.8</td>
<td>8.36</td>
<td>58.8</td>
<td>67.16</td>
<td>32.83</td>
</tr>
</tbody>
</table>
Figure 3.8: Residence and transition time percentage subplots at different flow velocities.

Table 3.7 shows the percentage of the cycle spent in each transition and residence zone based on the time divided by the total time for one cycle. The total time percentage of dwell time in the target temperature zones was reduced to 22% of the cycle at 15mm/s and 20mm/s. Figure 3.8 also showed the trend of each residence and transition time percentage as a function of flow velocity; the residence time percentage was monotonically decreased as transition time percentages increased. These numbers may help understand the DNA cocktail temperature distribution and predict or explain the experimental amplification results.

3.1.5: Thermal and Fluidic Simulation with a Substrate and Comparison to Results without a Substrate

In the CFPCR experiments, the CFPCR chips were made by hot embossing and the microchannel was on a polycarbonate substrate. The single microchannel simulation assumed vertical symmetry about the horizontal plane in the center of microchannel. Simulations were used to investigate difference between the steady-state temperature distribution for the
symmetrical model system and the actual device with the substrate, because the substrate acts as a heat conduction path and thermal capacitance.

The physical difference between two cases was that there was an additional 1 mm thick piece of polycarbonate below the microchannel. With the substrate, the system was not symmetric and the full height of the 150 µm×50 µm microchannel was used in the simulation. Natural convection was applied as a boundary condition at the top and bottom surfaces (15W/m²K). The material properties of air, water, and polycarbonate were the same as those applied in the symmetrical simulation (Table 3.1).

Figure 3.9 shows the temperature difference between the two models at the baseline 2 mm/s flow rate. The model that took the substrate into consideration had a slightly slower thermal response than the symmetric one and its renaturation temperature was not as low as for the symmetric one device. The difference contributed to increased thermal capacitance.

The differences are summarized in Table 3.8. The substrate led to shorter denaturation and renaturation times. The thermally critical point in the CFPCR was the temperature decrease between denaturation and renaturation. Natural convection was used in both designs to cool the device.

![Figure 3.9: Temperature distribution comparison with and without a 1 mm polycarbonate substrate.](image)
Table 3.8: Comparison between single channel simulation with and without 1mm thick substrate.

<table>
<thead>
<tr>
<th></th>
<th>Total Residence Time (s)</th>
<th>Total Residence Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>72-95 (s)</td>
<td>95 (s)</td>
</tr>
<tr>
<td></td>
<td>With substrate</td>
<td>1.358</td>
</tr>
</tbody>
</table>

The polycarbonate between each airpocket acted as a polycarbonate fin that dissipated heat more quickly than the neighboring airpockets because the thermal conductivity of polycarbonate (0.2 W/m k) was much higher than the thermal conductivity of air (0.0263 W/m k). In Figure 3.10(a), there was a sharp temperature gradient at 2 cm from the inlet and that corresponds to the position of the second polycarbonate fin in the airpocket layer. In Figure 3.10 (b), the same color band was a little wider than that in the airpockets illustrating the higher thermal resistance of air. The heat from the microchannel would flow though the polycarbonate fins to the environment faster than through the airpockets.

Figure 3.10: The temperature distribution and gradient at the polycarbonate fin between renaturation and extension. (a) The sharp temperature drop at the end of renaturation zone (68 °C). (b) The thermal contour of polycarbonate fin between two airpockets.
3.2 Microfabrication and Assembly of the CFPCR

3.2.1 LIGA Microfabrication CFPCR Mold Insert

Figure 3.11: The LSU CFPCR mold insert fabricated using LIGA microfabrication technology. (a) Full mold insert. (b) Close up of channel walls on the mold insert.

LIGA is a German acronym for lithography, electrodeposition and plastic molding [W. Menz et. al, 1991]. This technology uses synchrotron X-ray radiation as a lithographic light source and can be used to produce mold inserts with high aspect ratios, and smooth and vertical sidewalls.

A LSU CFPCR mold insert made by Mitchell [Mitchell et al., 2002] was available and is shown in Figure 3.11. The nickel microstructures were electrodeposited on a stainless steel substrate. This mold insert could be used for mass production of CFPCR devices embossed on polymer substrates.

3.2.2 CFPCR Hot Embossing and Thermal Bonding

Hot embossing was used for mass production of polymer chips. A PHI (City of Industry, CA) press with a vacuum chamber (Figure 3.12) was used for making replica polymer chips. Thick polycarbonate, 5 mm (Goodfellow, UK), was chosen as the CFPCR device material because of its low thermal conductivity, for discrete temperature distribution, and high glass transition temperature, for use in PCR denaturation. Circular-shaped polycarbonate plates with 5 inch diameter were cut for use in the hot embossing machine.
Polycarbonate plates were cleaned using isopropyl alcohol (IPA), deionized water (DI water), compressed air and then placed in an oven at 80°C overnight to bake out excess moisture and any residual monomer. Each plate was then placed in a vacuum chamber and held in place using an aluminum retaining ring with screws. The mold insert was mounted on the opposite fixture and maintained at a temperature of 185°C using independent thermocouples and heaters. The upper plate of the press was at 180 °C and lower plate at 120 °C enclosed the fixture with the polycarbonate plate and fixture at 180 °C with mold insert. After 4 minutes at 900 psi pressure, the press was released and the CFPCR chip demolded. The conditions for hot embossing were developed by the staff at the Center for Bio-Modular Microsystems at Louisiana State University (www.lsu.edu/cbmm) through trial-and-error.

After hot embossing, the microstructures on the polycarbonate were checked using a microscope to make sure the embossing results were satisfactory. After passing inspection, holes were drilled for injecting reagents and the embossed substrates bonded. There were two methods of annealing the chip. The first used the hot embossing machine and the second used a programmable convection oven. The condition for bonding a 250 µm thick polycarbonate coverslip was 157°C-159°C for the upper plate, lower plate, and holding fixture was 160 psi for 10 minutes. The second method in the oven used two pieces of glass of similar size to the
chips and paper clamps to secure the chips. The temperature profile of the oven was ramped to 150°C, maintained for 20 minutes, and then cooled down slowly.

![Figure 3.13: An SEM photograph of microchannels on the polycarbonate. The microchannel was 50 µm wide.](image)

**Figure 3.13**: An SEM photograph of microchannels on the polycarbonate. The microchannel was 50 µm wide.

![Figure 3.14: Cross-sectional views of the sealed microchannel after hot embossing.](image)

**Figure 3.14**: Cross-sectional views of the sealed microchannel after hot embossing.

An SEM picture of the microchannels embossed in the polycarbonate is shown in Figure 3.13. Figure 3.14 presents two cross-sectional views of the microchannel after thermal bonding, showing that the channel cross-section was unaffected by the bonding and the seal tight.

### 3.2.3 CFPCR Device Assembly

After annealing, the CFPCR chip was cut to the same size as the airpocket, 10cm long by 6cm wide, for assembly. The left hand side of Figure 3.15 shows the CFPCR with coverslip, thermocouples for each temperature zone and commercial thin film heaters (5W,
33 VDC, K0.0030C5, Watlow, Columbia, MO). The right hand side shows the airpocket (90mm × 75mm) which overlays the CFPCR chip.

![Figure 3.15: The assembled CFPCR.](image)

### 3.3 Limiting Performance of CFPCR

The purpose of this study was to determine the limiting performance and fastest permissible flow rate of the LSU CFPCR that led to successful amplification. Flow rates from zero to 20mm/s were evaluated and amplified DNA collected at the outlet for comparison.

#### 3.3.1 Experimental Apparatus and Methods

This research work was a collaboration between the Department of Mechanical Engineering and Department of Chemistry; Dr. Hashimoto from the Department of Chemistry finished the major part of the experiments.

CFPCR chips were prepared and a syringe pump (Harvard 22, Harvard Apparatus, Holliston, MA) was used to drive the PCR mixture through the spiral micro-channel. A glass syringe (Hamilton, Reno, NV) with a syringe-to-capillary adapter was used to make the connection between the pump and the microfluidic device. Temperatures were maintained during operation using electrical resistance heaters (KHLV-101/10, Omega Engineering, Inc.,
Stamford, CT) under closed loop PID control (CN77R340, Omega Engineering, Inc.,
Stamford, CT). Temperature feedback was through Type K thermocouples (5TC-TT-K-36-36,
Omega Engineering, Inc., Stamford, CT) mounted between the cover plates and heaters.

Either a 500 bp or 997 bp DNA fragment was individually amplified with a common
forward primer (5’-GATGAGTTCGTGTCCGTACAACTGG-3’) and different sequences of
the reverse primers (5’-GGTTATCGAAATCAGCCACAGCCACAGGC-3’ for the 500 bp fragment;
5’-GTACCTTTGTCTCTACGGCAACCTG-3’ for the 997 bp fragment). The forward
primer was labeled with IRD800 (Li-COR, Lincoln, NE) at its 5’-end for post-PCR
fluorescence detection.

Table 3.9: The chemical composition of DNA cocktail.

<table>
<thead>
<tr>
<th>Component</th>
<th>Tris-HCl</th>
<th>2X BSA</th>
<th>KCl</th>
<th>MgCl₂</th>
<th>dNTP</th>
<th>Primers</th>
<th>Template</th>
<th>Taq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>100 mM</td>
<td>0.5 mg/mL</td>
<td>50 mM</td>
<td>1.5 mM</td>
<td>200 mM</td>
<td>0.2 mM</td>
<td>0.1-10 ng/mL</td>
<td>0.07 U/mL</td>
</tr>
</tbody>
</table>

The PCR cocktail consisted of 10 mM Tris-HCl (pH 8.3), 2xBSA (0.5 mg/mL), 50 mM KCl,
1.5 mM MgCl₂, 200 mM of each dNTP, the primer pair (0.2 mM each), 0.1–10 ng/mL
bacteriophage l-DNA as the PCR template, and 0.07 U/mL Taq DNA polymerase. The
template was thoroughly denatured by heating to 95°C for 2 min prior to addition of the PCR
cocktail.

Before pumping the PCR cocktail through the micro-channels, the channel was
washed in sequence by pumping distilled water followed by 1X PCR buffer containing
2xBSA. The PCR mixture containing the template was then pumped through the chip at the
appropriate linear velocity with the PCR products collected into a microfuge tube from the
capillary outlet of the chip. Nominally, the temperature of the denaturation zone was set at 95
°C, and the temperature of the extension zone was set to 72°C. Active heating was not applied
to the renaturation zone. The collected samples were analyzed using a 5.5% crosslinked polyacrylamide gel. Fluorescence from the product was imaged by the Global IR2 DNA analysis system (Li-COR, Lincoln, NE) and the resultant band integrated over each separation lane with ImageQuant software (Amersham Biosciences, Piscataway, NJ). As a reference, the same PCR mixture used for the CFPCR device was run on a commercial thermal cycler (Techne, Burlington, NJ) using the same number of cycles (20).

3.3.2 Experimental Results and Analysis

The PCR products generated from the micro-CFPCR device at flow velocities of 1, 2, and 3 mm/s are shown in Figure 3.16. The results demonstrated that about 25%, 20%, and 10% of the reference PCR product yield was obtained for flow velocities of 1, 2, and 3 mm/s, respectively, using the CFPCR device. A total of 20 thermal cycles with the loops possessing a mean time ratio between 1:1:3 and 1:1:4 for each step of the PCR process (denaturation:renaturation:extension) were executed, with the exact ratio depending on the location of each loop (1:1:3 in the outer most loop). The 20 cycles were completed in 25.9, 12.9, and 8.6 min with cycle rates of 77.9, 38.9, and 25.9 s/cycle, respectively, for the three flow velocities used here, while approximately 50 min was required for the reference cycler to perform 20 thermal cycles (30 s denaturation, 30 s renaturation, and 40 s extension). The product yield for the CFPCR device was about 25% that obtained using the conventional thermal cycler at the lowest linear velocity used (1 mm/s) that approximately provides 14 s, 14 s, and 49 s for the denaturation, renaturation, and extension, respectively. The lower product yield was due to partial deactivation of the Taq polymerase by adsorption to the high surface-to-volume ratio PCR micro-channel (PC) and/or insufficient residence time in each temperature zone.
Figure 3.16: Effect of the flow velocity on the product yield. (a) Fluorescence images of the PCR products in the 5.5% polyacrylamide gel matrix. Lanes 1 and 5: DNA size markers. Lanes 2–4: CFPCR products at flow velocities of 1, 2, and 3 mm/s, respectively. Each PCR product was diluted 10-fold in TE buffer, then mixed with loading dye. The solution was denatured at 95°C for 2 min, then cooled on ice before gel loading.

For some applications, it might be important to obtain a detectable amount of PCR product quickly from a low copy number sample. Therefore, a study was carried out to establish the minimum copy number of template that could be detected when amplified through 20 thermal cycles using the CFPCR at a linear velocity of 10 mm/s (7.8 s/cycle and 2.6 min for completing the reaction). The results of these studies are depicted in Figure 3.17. The minimum template concentration that could be detected at 10 mm/s was 1 ng/µL, which corresponded to approximately $2 \times 10^7$ copies. Although no visible product band was apparent for 0.1 ng/µL of template, this did not necessarily mean that no product was generated at those concentrations. Most likely the amount of product produced under these PCR conditions was smaller than the detection limit of the fluorescence scanner associated with the gel. The PCR products were labeled with a near-IR dye molecule at the forward primer’s 5’-end, which typically improved detection sensitivity by 20X compared to conventional
DNA staining with ethidium bromide [Burns et al., 1996]. Random incorporation of plural numbers of fluorescent dye molecules into the PCR product during the reaction may provide improved sensitivity when fast PCR is required compared to the single 5’-end labeling employed here.

Figure 3.17: Fluorescence intensity of the PCR product gel band as a function of the input DNA copy number. A linear velocity of 10 mm/s was used. Each PCR solution was mixed with loading dye, then denatured at 95°C for 2 min, followed by cooling on ice prior to gel loading. Fluorescence images of the PCR products in the 5.5 % polyacrylamide gel matrix. Lanes 1 and 7: DNA size markers. Lanes 2–6: continuous-flow PCR products at various input DNA copy numbers, 10, 1, 0.1, 0.01, and 0.001 ng/µL, corresponding to $2 \times 10^8$, $2 \times 10^7$, $2 \times 10^6$, $2 \times 10^5$, and $2 \times 10^4$ copies/µL, respectively.

Figure 3.18: Linear velocity effects for the generation of a 500 bp PCR fragment. The identical concentration of input DNA molecules (10 ng/µL) was used for all CFPCR results. Each solution was mixed with loading dye, then denatured at 95°C for 2 min, followed by cooling on ice before gel loading. Lanes 1 and 5: DNA size markers. Lanes 2–4: CFPCR products at velocities of 10, 15, and 20 mm/s, corresponding to cycle rates of 7.8, 5.2, and 3.9 s/cycle, respectively.
PCR runs were next performed with a template concentration of 10 ng/µL to determine an upper bound on the linear velocity that could be used using the micro-CFPCR for the generation of either a 500 or 997 bp fragment. Figure 3.18 shows PCR results for the 500 bp fragment at linear velocities of 10, 15, and 20 mm/s.

The product band was detectable up to a flow velocity of ~15 mm/s, which allowed completion of 20 PCR cycles in only 1.7 min with a per cycle time of 5.2 s. For the 997 bp fragment, the maximum linear velocity that could be used to produce a detectable product was ~8 mm/s, giving a total reaction time of 3.2 min with the cycle time equal to 9.7 s (see Figure 3.19).

Figure 3.19: Linear velocity effects on the generation of a 997 bp PCR fragment. The PCR reactions were carried out using the same conditions as in Figure 7a except for the sequence of the reverse primer. Lanes 1 and 6: DNA size markers. Lanes 2–5: continuous-flow PCR products at linear velocities of 6, 8, 10, and 15 mm/s, corresponding to cycle rates of 12.9, 9.7, 7.8, and 5.2 s/cycle, respectively.

It was difficult to conclude from this data whether those cycle times (5.2 and 9.7 s for the 500 and 997 bp fragments, respectively) represented the fundamental limit of PCR, the residence time in the extension zone below the kinetic rate of the polymerase enzyme, or were due to the limitation of generating detectable amounts of product for the fluorescence gel reader. However, when the linear velocity was increased to reduce amplification time, the required cycle rate to generate product for PCR was primarily determined by the polymerase
extension time when thermal equilibrium was rapid as was the case for CFPCR. When differences in product yield were seen for two PCR fragments that differ only in size, the observed results could only be explained by the residence time in the extension zone approaching the kinetic limit for the PCR unless the primers differed in renaturing specificity since thermal equilibration effects on product yield were unaffected by product size. In addition, since the denaturation temperatures of the primer sets used for each PCR product were comparable, the deanturation/renaturation kinetics could be used to explain the product yield differences between these two fragments. As extension times were decreased below the kinetic limit for fully extending the fragment length defined by the forward and reverse primer pair, a failed PCR reaction will resulted. Therefore, under favorable thermal conditions, the fundamental limit on PCR time was highly dependent on the length of the product required.

3.3.5 Conclusion

A continuous flow PCR (CFPCR) device was microfabricated from polycarbonate and its performance as a function of reaction velocity was demonstrated. The ease of fabrication and the low cost associated with the device should made it particularly attractive for clinical diagnostic applications, where rapid PCR results were required. Finite element analysis (FEA) was used to help understand the heat transfer to the fluid mixture along a single channel and how the temperature distribution varied among different channel radii on the 20-cycle spiral CFPCR device. The transition and residence times of the fluid in each temperature zone were estimated to determine the effect of increasing flow velocity and changing heat transfer characteristics on the product yield. While the device was shown to produce product yields that were approximately 25% of a conventional block thermal cycler when operated with similar cycling times, the micro-CFPCR device could produce products at a much faster rate compared to conventional thermal cyclers. The CFPCR device could produce 500 bp fragments from λ-DNA in as little as 1.7 min (5.2 s/cycle) and a 997 bp
fragment in 3.2 min (9.7 s/cycle) with the limit on PCR time set by enzyme kinetics (Taq polymerase).

3.4 Liquid Crystal Application of CFPCR

To understand temperature distribution in the renaturation zone and validate the single straight microchannel simulation described in Section 3.1.1, thermochromic liquid crystals (TLCs) were chosen for visualization.

3.4.1 Introduction and Background to Thermochromic Liquid Crystals

The investigation of TLCs was by Prof. Terry Jones at Oxford University in 1985 [Jones et al, 1992]. Liquid crystals are used to describe an intermediate phase between solid and liquid. TLCs displayed many useful optical properties because of variation of orientational order following local temperature change [Hay et al, 1996]. The reflectivity wavelength of the liquid crystal molecules depended strongly on the local temperature within a specific range and its wavelength decreased with increasing temperature. The color at the lower starting point was red and the color changed to yellow, green, and blue as the temperature was increased. Liquid crystal molecules were micro-encapsulated by a nonreactive polymer for protection from solvents, UV radiation, high temperature, contamination, and for longer life. In practical applications, a calibration curve has to be obtained for interpreting surface temperature distributions from digital images of an investigated surface. There are different equations for transferring digital images from the red, green, and blue domain (RGB) to hue, saturation, and value domain (HSV). Each color has an unique wavelength, or hue, and it could be used as an index to tell different colors.

Since TLCs had the unique optical property of displaying different colors for different temperatures, there have been many different research directions. Some focused on fundamental thermal investigations like convection coefficient measurement [Camci et al, 1992] [Praisner et al, 2001] and some studied the behavior, calibration, and hysteresis phenomenon of the TLCs.
Camci [1992] developed a hue capturing technique for quantifying liquid crystal images. A hue versus temperature calibration curve was possible to obtain by using image capturing, image processing, and a thermal measurement instrument. The resulting calibration curve could be used for quantifying the surface images taken using the same equipment.

A discussion of errors that might affect liquid crystal results extracted from experiments and publications was performed by Wiberg [2003]. Possible errors were due to response time, aging, surrounding illumination disturbances, illumination angle, viewing angle, TLC thickness, digital resolution, image conversion, and noise. Data from the literature and also from their own experimental results were reviewed for potential thermochromic liquid crystal errors.

Three samples with different temperature ranges of micro-encapsulated thermochromic liquid crystals were used to investigate differences in response [Hay et al, 1998]. Although the TLCs had different temperature ranges, a dimensionless curve was found to fit all three liquid crystal temperature responses and the behavior of the liquid crystal had a similar mechanism over the different temperature ranges. These included expressions for the precision error as a function of the uncertainty of single pixels and the slope of the curve fit; the standard estimate of error used to describe the mean hue values after the curve fit; and the total uncertainty from the pixel error analysis and the curve fit error analysis.

Hysteresis was characterized by a decrease in reflectivity and a shift in the peak reflected intensity for each R, G, B component during cooling by natural convection [Anderson et al., 2004]. In a follow-up paper different heating and cooling starting points were used for investigating hysteresis behaviors [Baughn et al., 1999]. There was a significant hysteresis effect when the liquid crystal was heated much higher than the designed upper limit temperature. Using a separate calibration curve for each heating or cooling cycle was the best way to establish the relationship between temperature and hue since the
behaviors of the liquid crystal in heating or cooling were different. There was also a permanent decrease in reflectivity and a shift of the reflected peak intensity when the liquid crystal was exposed to extended higher temperatures. Hysteresis phenomena were the result of the crystalline structure of the liquid crystal changing.

Liquid crystals were also affected by the viewing angle and light source angle [Wiberg et al, 2003]. Different calibration curves were obtained using different angles between the lighting and viewing directions [Smith et al, 2001]. On-axis arrangement of the lighting yielded a smaller spatial standard deviation in hue than the off-axis arrangement, but the off-axis approach was easier to implement and could eliminate parallax distortion. Chaudhari [1998] pointed out that a 30° off-axis alignment had the best signal-to-noise ratio.

This group used liquid crystals to examine the temperature distribution on a microchamber PCR made on a silicone substrate. They studied the inside chamber temperature, and the temperature distribution all over of the device with and without different sizes of paper clamp, which were used to sandwich the system. A simulation was used to understand the thermal response at the center point of the chamber and the wall with an attached heater. There were 18 vessels on a silicon chip and each micro chamber was 2 µl. The temperature difference between the chamber center and the chamber wall was 0.3 °C in the renaturation zone (72°C), and 0.6 °C in the denaturation zone (95°C), based on large clamp contact area. Results for a smaller clamp contact area were 0.2 °C for renaturation and 0.4 °C for denaturation.

3.4.2 Experimental apparatus

This experiment was separated into two parts. The first step was to obtain a calibration curve, a relation between temperature and hue, which could be used for converting the surface applied liquid crystal image to hue. The second step was an investigation of the surface temperature distribution over the CFPCR device.
Figure 3.20 shows the experimental apparatus. It included a PC with software (Pinnacle Studio, V 9.0, Pinnacle System, CA) which could operate a digital camera (Panasonic GP-KR222) and store images, a gooseneck light source (Alpha 1501 Fiber Optic Illuminator with Dual Goosenecks, Microscopes USA, GA) with adjustable light intensity and light direction for the sample, a microscope with a stage for focusing the image surface and supporting the jig carrying the investigated CFPCR chip, a power supply for heating, a thermal controller (Series 96, Watlow, MO) for feedback control, and a thermometer (HH306, Omega Engineering, Inc., Stamford, CT) for monitoring the temperature. All experiments were carried out during the evening to avoid unnecessary disturbances since the liquid crystal were easily affected by light intensity, light angle, and other light sources, such as ambient and UV light. The experimental setup including light density, lighting angle, the sample position and elevation, was kept as constant as possible including the light intensity, light angle, and environment, for all experiments.

3.4.3 Process of Obtaining a Calibration Curve

In the first experiment, an alumina block (5 cm× 5 cm× 8 mm) was used for obtaining a calibration curve between temperature and hue. Thermal conductivity of alumina was high
(247 W/mK) so the temperature distribution was assumed uniform. From a numerical thermal model, the temperature difference over the alumina block was 0.159°C, with natural convection applied on the all of the surfaces with a coefficient 15 W/m²K as a boundary condition. The alumina block was polished with sand paper for smoothness and a black paint (SPB-250g, Hallcrest, IL) was sprayed, at roughly 20 psi, on the alumina block surface for the TLC background. The thermochromic liquid crystal (SPN-R50C20W, Hallcrest, IL) was airbrushed on after 20-45 minutes of drying of the black background paint. The temperature range of the TLCs offered from Hallcrest was from 50°C to 70°C but the calibration process was necessary to determine the exact temperature range for the experimental apparatus.

A jig was used to hold the alumina block on the microscope stage to make sure its position was consistent during all experiments. A leaf, K-type thermocouple (88309K, Omega Engineering, Inc., Stamford, CT), specially designed for surface temperature measurement, was positioned on the liquid crystal via a thermal paste to monitor its temperature while a thin-firm heater (KHLV-102, Omega Engineering, Inc., Stamford, CT) was applied as a heat source. A gooseneck light source was adjusted for a 30 degree incline from the measurement surface to obtain the best signal-to-noise ratio (Chaudhari. et. Al, 1998). The temperature distribution across the thermocouple was assumed uniform because the leaf thermocouple was small (3.2mm) compared to the alumina block (5 cm× 5 cm× 8 mm).

The experiment started from 51.1°C and the images was taken after waiting several minutes to ensure a stable temperature distribution. The increment was 0.3-0.5°C depending on the sensitivity of color variety. The red color band was much smaller than the green and blue bands so the increment was 0.3°C at the lower temperatures. At higher temperatures, the blue color band was larger than the other two colors and it had the lowest sensitivity with the largest temperature increment for each step, 0.5°C. Each image was taken with a Panasonic digital camera operated with Pinnacle software installed on a PC. The images were stored on the PC hard drive for later processing. The digital camera was a Panasonic GP-KR222 and its
maximum sensing area was 6.4 mm×4.8 mm. The magnification of the objective was 1X (11-8216, Ealing, CA) and its working distance was 150 mm. Each image was captured as a three-dimensional matrix of red, green and blue values and stored into Tagged Image File Format (tiff) files. Each image had 640 × 480 pixels and 8-bit resolution for each color, so each image was 24-bits and 640 × 480 × 3 (0.9 MB). The captured image area was 6.4 mm × 4.8 mm and its position was very close to the thermocouple tip position, but did not include the thermocouple tip image to simplify later image processing. The area covered by each pixel in these images was 10 square micrometers (640 × 480 pixels with 6.4 mm × 4.8 mm). After taking all of the images for each temperature step, the images were converted to the hue domain using the unique peak wavelength of each color to obtain a relationship between temperature and hue. Reducing noise was an important task during image processing and the media filter command in Matlab (vers. 7.0, The MathWorks, Inc., Natick, MA) was used to reduce spatial noise in the images. The R, G, and B values of each pixel were replaced by an average of the neighboring 5 × 5 pixels. This filter could decrease the local noise from 3% to 0.9% (Behle M. et. al, 1996), so the filter command was used before domain transfer to achieve better precision.

Matlab (vers. 7.0, The MathWorks, Inc., Natick, MA) was used for correcting the raw data into the hue domain using Equations 3.4. The output of Equation 3.4 were compared with the results from Equation 3.5, which was suggested by Hay and Hollingsworth to have the average lowest uncertainty, and yielded a slightly different conversion (Wiberg et. al, 2003).

\[ \begin{align*}
\text{If } R &> G \text{ & } R > B & \quad H &= \frac{G - B}{6(R - \min(RGB))} \\
\text{Else if } G &> B & \quad H &= \frac{2 + B - R}{6(R - \min(RGB))} \\
\text{Else} & & \quad H &= \frac{4 + R - G}{6(R - \min(RGB))}
\end{align*} \] 

45
The 40 data points were used to construct a calibration curve using a fifth-order regression function in Malab (vers. 7.0, The MathWorks, Inc., Natick, MA). The temperature range was from 51.1°C to 69.5°C and Figure 3.21 shows the calibration curve. The lower temperature part of this calibration curve had a higher slope so that in the lower temperature red zone, sensitivity was much higher than in the other temperature zones.

Figure 3.21: The calibration curve for the liquid crystal giving a relation between temperature and hue.

Two major errors could occur during liquid crystal use, pixel precision error and calibration. Hay and Hollingsworth (1998) suggested using root-sum-square of the standard estimate of error (Eq 3.6, SEE) to obtain the calibration curve and the precision error from pixel to pixel (Eq 3.7, standard deviation) for the hue to measurement uncertainty.

\[
H = \arctan \left( \frac{\sqrt{3}(G - B)}{2R - G - B} \right) \quad \text{3.5}
\]

\[
\text{SEE} = \left[ \sum_{j=1}^{k} \frac{(T(h) - T_{j,1}(h))^2}{(k - j - 1)} \right]^{\frac{1}{2}} \quad \text{3.6}
\]

\[
\delta T = \frac{dT}{dh} \delta h \quad \text{3.7}
\]
A first approximation of the combined uncertainty considering the standard estimate of error (Eq 3.6) and the pixel-to-pixel standard deviation (Eq 3.7 is shown in Equation 3.8). The parameter, \( k \) in Eq. 3.6, was the number of data points and \( j \) is the order of the regression calibration curve fit; \( k \) was 40 points and \( j \) was 5. From these definitions, the SEE was 0.1894 °C and the average pixel-to-pixel standard deviation was 0.2195 °C over 40 points. The overall uncertainty was 0.4089 °C, which was 2.2 % of the useful range of 18.4 °C. Figure 3.22 shows the error bars when considering precision error at each data point (Eq. 3.11). The error bar increased when the slope increased because the temperature-to-hue ratio increased more rapidly so fewer hue scales were used to represent each degree Celsius, decreasing the sensitivity. Figure 3.23 shows the uncertainty envelope with the two dashed lines calculated from Eq. 3.8 along with the main calibration curve to predict the uncertainty for each point. The uncertainty in the high temperature range was slightly larger than that of the low temperature range due to natural optical properties of TLCs.

\[
\delta T = \left[ \left( \frac{dT}{dh} \delta h \right)^2 + (2 \times SEE)^2 \right]^{\frac{1}{2}} \tag{3.8}
\]

Figure 3.22: Pixel to pixel standard deviation error bar for each data point (Eq. 3.8).
3.4.4 Process for CFPCR Chip Temperature Distribution Experiments

The CFPCR chip was made by hot embossing, annealing, and assembly (Hashimoto et. al, 2004). A micro milling machine (Kern MMP – Microtechnic, Murnau-Westried, Germany) was used from the backside of the substrate to make a rectangle embedded in the CFPCR polycarbonate chip 4 cm long, 3 cm wide, and 1 mm thick to the left of microchannel. An airbrush was used to apply the black background paint (SPB-250g, Hallcrest, Glenview, IL) which was dried for 20-45 minutes at room temperature. The thermochromic liquid crystal was sprayed over the black paint and dried for another 20-45 minutes. Before setting up the CFPCR for an experiment, a syringe pump (Model 200, kd Scientific, MA) was used to inject water representing the DNA cocktail in the microchannel. The CFPCR was set on a Nikon MM-22 microscope stage. The experimental apparatus for the CFPCR test was the same as for the calibration, without moving any components because a different perspective on the liquid crystal would give different colors. Figure 3.24 shows a schematic of the airpockets layout. The captured image area was gray area and it was located in the middle of the renaturation zone.

Two or three different thermocouple tip positions were used to check the temperature
distribution in this area for both non-active heating cases and active heating cases. Figure 3.25 presents the different configurations of the feedback thermocouple tip positions. Each filled circle shows a different thermocouple tip position; the upper row contains the non-active heating cases, each with two measurement points, and the lower row shows the active heating cases, each with three thermocouple points. The goal was to study the thermal uniformity of the polymer chip in the renaturation zone, and the difference due to the different locations of the temperature measurement.

The distance from the innermost microchannel to the outermost microchannel was 5.75 mm and the size of the viewing area of the digital camera was 6.4mm, so that the temperature distribution access the 20 microchannels could be observed. After taking images, the color images were filtered, and converted to thermal contour.

![Figure 3.24: The layout of CFPCR chip with airpockets and the image captured area.](image)
Figure 3.25: Upper row was the non-active heating cases and lower row was the active heating cases. The filled circles in each configuration were the thermocouple tip positions.

3.4.5 Liquid Crystal Experimental Results and Discussions

Experimental results are shown in Figure 3.26 and Figure 3.27. The former shows the non-active heating cases with 2 feedback control points and Figure 3.27 summarizes the active heating cases with 3 feedback control points. The upper images in both figures were the raw images taken from the digital camera and the lower images were the temperature contours for each case after image processing. The temperature contours show how the temperature gradients varied depending on the feedback thermocouple positions. The bold line across the upper images was the path defined for assessment of thermal uniformity.

In Section 3.1 (Figure 3.7), a single microchannel model was used for thermal simulations to understand the temperature distribution along the microchannel. From the simulated steady-state temperature distribution (Figure 3.7), the lowest temperature along the channel was 68 °C without a heater in the renaturation zone and 68 °C was only reached at the midpoint of the microchannel in the renaturation zone. Natural convection was inadequate for dissipating heat from the device.

Table 3.10 displays the highest and lowest measured temperatures along the measurement line and their difference for each configuration. The lowest temperature
difference of 1.42 °C occurred in the second case under active heating. Three point feedback
gave better temperature uniformity compared to non-active controls since the independent
PID loop brought the better temperature control for each temperature zone. The temperature
distributions in Figure 3.27 for three active heating cases were almost blue because the
temperatures in all three cases were near the upper limit of the liquid crystal. On the other
hand, the temperature distributions in Figure 3.26 showed more significant temperature
gradients than Figure 3.27. In Figure 3.26, the right upper corner in the second case was black
because the temperature was lower than the liquid crystal minimum temperature of 51.1 °C.

In Figure 3.26, the difference between non-active and active heating cases was the
third thermocouple position for feedback control. Care had to be taken for investigating
Figure 3.26(a) and Figure 3.27(a), Figure 3.26(b) and Figure 3.27(b), Figure 3.26(c) and
Figure 3.27(c). In the (a) and (b) cases, the highest temperature was in the innermost
microchannel (left lower corner) and decreased toward the outermost microchannel (right
upper corner), while the highest temperatures were on both (c) cases. The heat transfer
direction in these three cases was not changed by the addition of a third thermocouple, but the
temperature distributions were more uniform.

Although the single microchannel simulation discussed in Section 3.1.3 gave 68°C as
the lowest temperature this device could reach without active heating in the renaturation zone,
the highest temperature in non-active heating cases from the liquid crystal image was 64.76°C.
There were several reasons for this difference. In the simulation, the boundary conditions
applied at the interface wall between the microchannels were insulated based on the
assumption that the heat could barely penetrate microchannels (z direction in the Figure
3.1(a)) because of the high thermal resistance compared to the thermal resistance in the y
direction (Figure 3.1(a)). From Figure 3.26, the heat flowed laterally across the core and the
microchannels were below it. The real boundary conditions for the microchannels were not
insulated, there was significant heat transfer across the 20 microchannels. The CFPCR chip
had circular spiral microchannel layout instead of single straight microchannel in the simulation. The thermal interactions introduced by the spirals microchannel layout, three-dimensional heat transfer, and heat transfer between conduction and convection were not fully considered completely in the single channel simulation. In the active heating cases, the highest temperature was 67.21 ºC while the temperature was set at 68 ºC in the controller. The thermocouple position was located on the bottom side, with liquid crystal applied on opposite (top) side, and the distance between the thermocouples and liquid crystal surface could also contribute to the difference.

3.4.6 Liquid Crystal Experimental Conclusions

Thermal management is still a critical issue in micro- even nano- scale microdevices, especially in the chemical and biochemical applications because of the temperature sensitivity of chemicals. Thermochromic liquid crystal is a widely-used tool for convection coefficient measurements because it enables measurements over a wide area in one image and are precise. In this experiment, the images were taken in the mid-section of the renaturation zone in order to investigate thermal uniformities of the polymer chips, the effects of different thermocouple positions, and validation of the thermal and fluidic simulations in the single straight microchannel. From the temperature distribution on the CFPCR chip surface, the temperature was not as uniform as assumed, compared to other common MEMS materials like silicon, because its thermal conductivity was low. Active heating could reduce this non-uniformity but still led to a 1.42 ºC difference (across the 20 channels). The temperature distribution was different from what was expected from the simulation. Potential contributions to the error could be three-dimensional heat transfer, the CFPCR circular microchannel layout, and complex heat transfer introduction between conduction and convection. The temperature contours for different configurations were more understandable and analysis because they showed the heat transfer direction and the temperature contour ranges among the all area. Adding the third thermocouple in the renaturation zone did not
change the heat flux direction but it did lead to better thermal uniformity compared to the two thermocouple, non-active heating approach.

Figure 3.26: Upper row are the thermochromic liquid crystal images for three different thermocouple tip positions with no heating in the renaturation zone. The red line across each image was the path defined for comparison. The lower row are the temperature gradient images after image processing for each thermocouple tip setup. (a): First setup. (b): Second setup. (c): Third setup.

Figure 3.27: Upper row shows the thermochromic liquid crystal images for three different thermocouple tip positions with active heating in the renaturation zone. The red line across each image was the path defined for comparison. The lower row presents the temperature gradient images after image processing for each thermocouple tip setup. (a): First setup. (b): Second setup. (c): Third setup.
Table 3.10: The temperature distribution and temperature difference are evaluated along the path for each liquid crystal image including non-active heating and active heating.

<table>
<thead>
<tr>
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<th>2</th>
<th>3</th>
</tr>
</thead>
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<td><strong>No heating</strong></td>
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<td></td>
<td></td>
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<td>6.7</td>
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<td><strong>Active heating</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Highest Temperature (°C)</td>
<td>65.38</td>
<td>63.81</td>
<td>67.21</td>
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<td>Lowest Temperature (°C)</td>
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<td>1.42</td>
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</tr>
</tbody>
</table>
Chapter 4: Development of the Shuttle PCR and Its Performance

4.1 Shuttle PCR Concept

4.1.1 Motivation for the Shuttle PCR

PCR micro devices can be separated into two kinds of devices, chamber and continuous flow devices. Although micro PCR devices have been developed for several years, many researchers are still studying new types of PCR, integrating different technology for better performance. Four key points must be evaluated in assessing PCR performance; the time to perform the reaction, power consumption, sample volume, and the ability to be integrated with other biochemical manipulations [Quake et. al 2002]. Based on CFPCR experimental results, the shuttle PCR was studied and developed.

The experimental results for the CFPCR showed that CFPCR performance could be improved within shuttle PCR design. The reagent consumption of the CFPCR was too large compared to micro, nano, even pico liter micro devices. The total length of the CFPCR including the preheat and postheat microchannels was 1.78 meters giving a volume of $1.335 \times 10^{-2}$ ml. The time needed for 20 cycles in the CFPCR (Section 3.3.2) for 500 bp was 1.7 minutes (5.2 seconds/ cycle) and it was almost at the chemical dynamical limit for the PCR reaction, but the shuttle PCR promised potentially faster reaction times.

4.1.2 Introduction to and Background for the Shuttle PCR

The lab-on-a-chip was developed in the early 1990s by Manz [Manz et al. 1990]. The purpose was to develop an integrated and powerful chemical analysis system on a chip. To achieve this goal, the first step was to study how to manipulate reagents moving in micro- or nano- channels, especially in very complex structures for multiple tasks on a single chip or integration with other function chips. Electrokinetic manipulation, vesicle encapsulation, and mechanical valves were three possible technologies [Hong et al. 2003]. Electrokinetic manipulation was widely applied for capillary electrophoresis; the basic idea was to obtain bulk fluid motion, electroosmosis, or motion of charged particles in the fluid, electrophoresis,
by applying a voltage difference between two reservoirs [Wong et al, 2004]. Vesicle encapsulation used two phase flow, air and liquid, in early applications, but the compressibility of air made it challenging to operate in complicated geometries. Reagent and oil systems were developed to replace air and fluid vesicle encapsulation application because of its controllability in a microchannel system [Song et al, 2003]. Mechanical valves were one of the most robust and independent ways to control the fluid, but fabrication and complexity induced from fabrication reduced practical applications.

The purpose of studying the shuttle PCR was to focus on the potential for minimizing reagent consumption, and easing integration. The shuttle PCR was a single microchannel because of the simplicity and ease of integration with other devices. Figure 4.1 and Figure 4.2 show the configuration of shuttle PCR. It was composed of a main microchannel, four reservoirs, four airpockets penetrating the chip to separate the temperature zones, and two branch microchannels. The two branch microchannels were for injecting DNA into the main microchannel for amplification.

![Figure 4.1: The layout of shuttle PCR.](image-url)
Electrophoresis was used to drive the DNA plug in the main microchannel of the shuttle PCR, which was divided into three steady-state temperature zones. The two ends of the main microchannel were at 95°C and 55°C, and the 72°C zone was in the central section. Each temperature zone was separated from the others by four low thermal conductivity airpockets, blocking heat conduction from the higher temperatures to lower temperatures. Each target temperature was achieved by feedback control in experiments. Figure 4.3 shows the three independent temperature zones and the two transition zones without enclosure for 95°C-72°C, 72°C-55°C, respectively. The preheating step could be achieved on this chip by applying a voltage to keep the DNA cocktail in the 95°C reservoir for total denaturation. DNA was moved from 95°C to 55°C through 72°C without stopping; reversing the applied voltage moved the DNA back to 95°C through 72°C for another cycle. In the last cycle, the DNA could be retained in the 72°C zone for final extension and then drawn from the sample reservoir located in 72°C zone for further analysis.

Thermal management is an important issue in MEMS applications, especially when processing chemical or biochemical reagents. The temperature must be as precise as possible to obtain better reaction performance. Liquid crystals were used for monitoring the temperature distribution over an area in the CFPCR, which had 20 microchannels and used one thermocouple to control the temperature in each zone. There was significant temperature variation across microchannels leading to potential lower reaction rates and yields. In the
shuttle PCR, only a single microchannel was employed for loading the DNA and that would be more amenable to thermocouple-based feedback control.

Figure 4.3: Three temperature blocks separated by four airpockets.

Air has very low thermal conductivity, 0.0263 W/m K, which makes it a very good insulator. The four airpockets penetrating though the chip offered good insulation against heat transfer from high temperature zones to low temperature zones and made three independent temperature zones. The distance between airpockets and the microchannel was 500 µm but the small central bridges connecting each temperature zone were small enough to prevent heat leaking.

**4.2 Electrokinetic Phenomenon**

Electrophoresis was chosen as the shuttle PCR driving force due to its integration ability. From the standpoint of geometry, this single microchannel layout could reduce problems in fabrication and integration. Electrophoresis was one of the most common fluid manipulation tools in micro scale devices.

**4.2.1 Electrical Double Layer**

Electrokinetic effects were first observed by Reuss in 1809 in an experimental investigation. In 1879, Helmholtz developed the electrical double layer (EDL) theory, which related the electric and flow parameters for electrokinetic transport [Karniadakis et al., 2000].
Most solid surfaces have electrostatic charges or an electrical surface potential when they are in contact with aqueous solutions. The electrostatic charges on the solid surface attract the counter-ions in the liquid and the concentration of counter-ions near the wall has more dense concentration than that in the bulk liquid. Due to the electrical repulsion, the concentration of co-ions near the wall is lower than that of the counter-ions. There is a net charge, caused by excess counter-ions, in the region very close to the wall and this net charge should balance the charge at the solid surface. The charged surface and the layer which has the balancing counter-ions are called the electrical double layer (EDL). Figure 4.4 is an illustration of the ionic concentration field in an electrical double layer. The charged surface, a thin layer, named stern layer or compact layer, is strongly attached to the surface and is immobile. The thickness of the stern layer is normally several Angstroms. The charge and potential distribution in this layer are determined by the geometrical restrictions of ions, molecule sizes, interactions between ions, and the solid wall properties.

In the diffuse layer next to the stern layer the net charge density is gradually reduced to zero, the net charge density of the bulk fluid. Ions in this area are mobile and its thickness is primarily dependent on the solution bulk ionic concentration and electrical properties. The thickness of this layer is from several nanometers, with high ionic concentration solutions, up
to a few micrometers with very low bulk ionic concentration. The boundary between these two layers is called the shear plane. The potential of the shear plane is referred to as the zeta potential, measured from experiment, and the potential of the solid wall is generally assumed to have the same potential because the real potential of the solid wall is difficult to measure experimentally.

Figure 4.5 shows the potential distribution from the solid surface to the bulk fluid. The solid surface has the largest potential and the shear plane has the most useful potential, the zeta potential. The potential distribution from the shear plane to the bulk fluid is described by the Boltzmann equation (Eq 4.1).

\[ n_i = n_i^\infty \left[ 1 - \frac{z_i e \Psi}{k_B T} \right] \]  

Where \( n \) is the ion number concentration, \( \Psi \) is the electrical field potential, \( z \) is the absolute value of the ionic valence, \( e \) is the fundamental charge of an electron, and \( k_B \) is the Boltzmann constant [Li, 2004].

4.2.2 Electroosmosis

Electroosmosis is the motion of a bulk fluid after a voltage is applied. The solid surface has a surface electrical charge when this surface is in contact with aqueous solution. The stern layer is immobile and the EDL is the moving layer when a voltage difference applied. The ions in the EDL move after applying a voltage difference, and the fluid molecules around the ions are also dragged in direction of motion and generate a bulk fluid motion. If the solid surface is negatively charged, the EDL will be positively charged. The positive ions in the EDL will move toward the cathode pulling the liquid molecules moving with them.

Electroosmosis is used to transport sample or mix different reagents because it can be employed by simply applying a voltage difference. The goal of MEMS is to develop a portable, multi-functional, easy-to-operate, and inexpensive device and electrokinetic flow
could be a solution for manipulating fluid movement in the micro scale.

![Figure 4.5: Illustration of the potential distribution in an electrical double layer. [Li,2004.]](image)

In convectional pressure-driven devices, a significant applied pressure is necessary for injecting and pushing chemical reagent flow through a narrow and long microchannel. Also, the parabolic flow profile increases the surface area for dispersion of the reagent. Electroosmosis can have better response for starting movement, ease of control of the fluid direction by switching the polarity of the applied voltage, a flat flow profile due to no fluid velocity gradients and potential fields outside the EDL, less dispersion, and a small back pressure.

Equation 4.2 describes the relationship between electroosmotic mobility and applied voltage. This equation is known as the Helmholtz-Scoluchowski equation and it is valid until the thickness of EDL is thin compared to the microchannel dimension. Homogeneous microchannel surfaces, a uniform zeta potential, and no pressure gradient are assumed. Heterogeneous microchannel surfaces and a non-uniform zeta potential may change the EDL, while the pressure gradient has a superposition effect on the flow field [Nguyen et al., 2002].

\[
\mu_{\text{eff}} = \frac{\varepsilon E \xi}{\eta} \tag{4.2}
\]

\[
k^2 = \frac{2z^2 e^2 n_w}{\varepsilon \varepsilon_0 k_b T} \tag{4.3}
\]

Where \(\mu\) is the mobility, \(E\) is the applied voltage, \(\xi\) is the zeta potential, and \(\eta\) is the dynamic viscosity in Equation 4.2. And \(k\) is the Debye-Huckel parameter, \(z\) is the
absolute value of the ionic valence, $e$ is the fundamental charge of an electron, $n$ is the ion number concentration, $\varepsilon$ is the dielectric constant, $k_b$ is the Boltzmann constant, and $T$ is the absolute temperature in the Equation 4.3.

4.2.3 Electrophoresis

Electrophoresis is another electrokinetic phenomenon. Charged particles suspended in a bulk liquid will move toward the anode or cathode depending on their charges after applying a voltage difference. Theoretically, the bulk liquid is stationary while the charged particles are moving under the applied electrical field. During the movement, the charged particles are accelerated by the electrical body force which is gradually balanced by viscous drag force generated by the bulk fluid.

The most common application of electrophoresis is the separation of large molecules, like DNA or proteins in gel matrices. Electrophoresis was first introduced by A. W. K. Tiselius in 1937 and he was awarded a Nobel Prize in 1948 [Breuer, K. S. (In press.), 2004]. The basic idea of capillary electrophoresis is based on different mobility of the different size DNA fragments. Shorter DNA will move faster than the longer DNA in the viscous gel matrices and can be separated from those longer DNA fragments. Taking advantage of this difference in mobility of different DNA, each specific DNA can be separated and marked based on its detection time.

In principle, electrokinetic flow is a better tool to manipulate chemical reagents on micro scale. In practical, electroosmosis and electrophoresis happen at the same time. In most cases, the microchannel solid wall, reagent solution, and charged particles have their own charge. In this study, DNA is the target chemical reagent to be amplified in the PCR device and its cocktail is also a charged solution. When a voltage is applied, the DNA, negative charged large molecules, will move toward the anode while the cocktail is moving in the opposite direction due to the negatively charged polymer surface. The direction of the DNA movement is dependent on the superposition of the DNA molecule mobility and the
electroosmosis mobility.

4.2.4 Joule Heating

Application of a voltage in a channel leads to Joule heating due to the resulting axial current through the microchannel. The bulk liquid temperature will increase and the magnitude of the increase depends on the time and current. The temperature field changes and the temperature dependent liquid properties will also change. All fields including the temperature field, flow field, and electrical field should be considered simultaneously because of the change of temperature dependent electrical conductivity, thermal conductivity, and viscosity of the liquid [Xuan et al., 2004].

Without Joule heating, the temperature of the bulk liquid should be the same as the environment, if the chemical reagent was assumed to have no heat generation. Dongqing Li and his colleagues [Li, 2004] studied the Joule heating effects on the temperature field, flow field, and electrical field, using numerical simulation and theoretical analysis. Temperature increases, concave/convex flow profile, increased flow rates, lower capillary electrophoresis efficiency, and increased dispersion were caused by Joule heating. The Joule heating dissipation depends on radial heat transfer into the surrounding environment so the amount of heat accumulated depends on how fast heat generation can be dissipated to the microchannel walls. Temperature dependent fluorescence was used to monitor the temperature increase caused by Joule heating [Ross et al., 2001]. Two different kinds of materials having different thermal conductivities, fused-silica capillaries and acrylic microchannels, were used to measure the temperature variation and it was concluded from the experiment results that the fused-silica capillary had better capability to reduce heat accumulation due to its higher thermal conductivity. Higher thermal conductivity can transfer internal heat from the bulk liquid to the surrounding environment faster.

Changing chemical composition is another method of decreasing heat generation from large currents [Chen et al., 2005]. KCl is a basic component in a typical PCR kit but it
increases the Joule heating significantly. Chen used different concentrations of KCl, even no KCl, to investigate its effects while monitoring the current through the microchannel. The experiment showed that currents from 379.3 µA with 50 mM KCl buffer could be reduced to 66.3 µA with no KCl buffer based on an applied 500 V/cm electrical field.

4.3 Numerical and Theoretical Simulation of a Shuttle PCR

4.3.1 Three-Dimensional and Steady-State Thermal Simulation for the Shuttle PCR

Thermal numerical simulations were used to understand the temperature distribution in the shuttle PCR. The schematic of the shuttle PCR was described in Section 4.1.2. The boundary conditions applied for this model were natural convection (15 W/m² K) for all surfaces except the bottom surfaces, which modeled heating with a uniform heat flux. The temperature dependent water properties (Incropera et al., 2002) were applied in the microchannel and the polycarbonate properties were from Goodfellow, the vendor of our polycarbonate (Table 3.1). Figure 4.6 shows the temperature contour of the shuttle PCR. The nominal temperatures for the shuttle PCR were 95°C, 72°C, and 55°C from the left side block, middle block, and right block, respectively. The thermal conduction was blocked by air insulators to reduce heat transfer between zones.

This would enable individual target temperature controls for each zone; especially for different DNA fragments which had different renaturation temperatures from 50°C to 70°C. The length of the zones was 4 mm for denaturation, 6.5 mm for extension, 3 mm for renaturation, and 1 mm for two transition zones.

4.3.2 Transient Thermal Simulation using ANSYS

Transient thermal simulations were used to understand the device thermal performance over time. The boundary conditions and material properties applied in the transient thermal simulations were the same as the conditions applied in the steady-state simulations.
Figure 4.6: The model for 3-D thermal simulation of the shuttle PCR.

The constant heat flux as applied for the denaturation, extension, and renaturation zones were 1505 W/m² K, 970 W/m² K, and 620 W/m² K, respectively. The power applied for each zone was 0.097825W, 0.06305W, and 0.0403W in ANSYS (vers. 8.1, ANSYS, Inc., Canonsburg, PA). Figure 4.7 shows the temperature as a function of time for each zone. The time needed for the shuttle PCR to reach steady-state was about 350 seconds. The heat transfer direction in the shuttle PCR or each specific part, microchannel or each temperature block, could be understood from transient simulation.

4.3.3 Analytical Thermal Analysis for the Shuttle PCR

Considering each temperature block as an independent temperature zone, the relevant heat transfer phenomena for each block was convection around the extended surfaces, external power going into the block, and the heat capacity of this block. Before describing these phenomena, the Biot number was calculated for each temperature zone to determine whether the temperature of the block was uniform or not. Equation 4.4 was used to calculate the Biot number. It was the ratio of conduction thermal resistance to convection thermal resistance.

\[
Bi = \frac{hL_C}{K} = \frac{R_{\text{cond}}}{R_{\text{conv}}} \tag{4.4}
\]

\[
L_C = \frac{V}{A_S} \tag{4.5}
\]
Figure 4.7: Transient response for each zone with a constant heat flux input.

Where \( Bi \) was the Biot number, \( h \) was convection coefficient (W/m\(^2\) K), \( L_c \) was the characteristic length (m), \( K \) was the thermal conductivity (W/ m K), \( R_{\text{cond}} \) was the conduction thermal resistance, and \( R_{\text{conv}} \) was the convection thermal resistance. In the equation, \( V \) was the volume of this block and the \( A_s \) was the surfaces.

The Biot numbers for the three temperature zones were the same because they had the same dimensions. The volume of each block was \( 6.5 \times 10^{-8} \) m\(^3\) and surface was \( 1.63 \times 10^{-4} \) m\(^2\). The characteristic length was \( 3.98 \times 10^{-4} \) m and the Biot number was 0.0299; much less than one, so the convection thermal resistance dominated and the conduction resistance inside the polycarbonate block was ignored. The assumption was that there were no thermal gradients inside the polycarbonate blocks.

The power input from power supply was selected to be equal to the sum of the increase of the polycarbonate block heat capacity and convection dissipation. Since the temperature of each block was assumed to be uniform based on the small Biot number, the conduction was not considered in determining the required power.

\[
P = m \frac{dT(t)}{dt} + hA[T(t) - T_{\text{inf}}]
\]  

4.6
Where \( P \) was the power supplied by power supply (Watt), \( m \) was the block mass (Kg), \( T(t) \) was the temperature of his block (K), \( h \) was the natural convection coefficient (W/m\(^2\)K), \( A \) was the surfaces (m\(^2\)), \( T_{\text{inf}} \) was the ambient temperature (K).

Matlab (vers. 7.0, The MathWorks, Inc., Natick, MA) was used to solve Equation 4.6 using all of the dimensional and material properties. The ODE 45 solver in Matlab was used to obtain the solution and then the results plotted against time. Figure 4.8 shows a plot of three independent block temperature distributions against time. The time needed for reaching the target temperature was roughly about 350 seconds which has similar time constant from ANSYS thermal simulation (Figure 4.7).

The power needed for denaturation, extension, and renaturation were 0.1073W, 0.0735W, and 0.0485W, respectively; from the analytical model, these were different and higher than those obtained using the ANSYS thermal simulation, 0.097825W for denaturation, 0.06305W for extension, and 0.0403W for renaturation. The reason for this difference were the simplifying assumptions made for the analytical analysis.

![Figure 4.8: The transient temperature distribution for each block using analytical heat transfer equation.](image-url)
The independent block was assumed to have a uniform temperature based on the small Biot number, but the practical temperature was not uniform over each block. Conduction between blocks was also neglected.

Table 4.1: The power demand for three temperature zones using numerical simulation and analytical simulation.

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<th>Denaturation</th>
<th>Renaturation</th>
<th>Extension</th>
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<td>Numerical Simulation from ANSYS (W)</td>
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<td>0.06305</td>
<td>0.0403</td>
</tr>
<tr>
<td>Analytical Simulation and result (W)</td>
<td>0.1073</td>
<td>0.0735</td>
<td>0.0485</td>
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</table>

4.4 Thermal and Molecular Diffusions

Diffusion is a problem in electrokinetic flow. There are many kinds of diffusion, thermal diffusion, molecular diffusion, hydrostatic diffusion, and diffusion induced from the opposing direction EOF. These all happen simultaneously and make electrokinetic flow behavior more complicated.

4.4.1 DNA Macromolecular Mobility

DNA are negatively charged macromolecules and the direction of movement is toward the anode under an application potential difference. DNA mobility in free solution, for single strand or double strands, is almost the same except for short DNA. DNA mobility is different in different electrolytes. The free solution of DNA mobility in TAE (Tris-acetate-EDTA) was measured to be \((3.75\pm0.04) \times 10^{-4} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}\) at 25°C while the mobility in TBE (Tris-borate-EDTA) was \((4.5\pm0.1) \times 10^{-4} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}\) under the same conditions [Stellwagen et al., 1997]. This result was valid from 400 bp to 48.5 kb and the mobility in TBE was 20% larger than that in TAE.

4.4.2 DNA Molecular Diffusion

A model was needed to understand the relationship between temperature, diffusion,
mobility, and different base pair sizes. Equation 4.7 was the electrophoretic mobility ($\mu$) in terms of total charge ($Q$) and friction factor ($f$) and equation 4.8 was the Einstein equation. Combining Equations 4.7 and 4.8 produced to Equation 4.9 which was a relation between mobility and diffusion coefficient.

$$\mu = \frac{Q}{f} = \frac{Ze_0}{f} \quad \text{(4.7)}$$

$$f = \frac{K_BT}{D} \quad \text{(4.8)}$$

$$\frac{\mu}{ZD} = \frac{e_0}{K_BT} \quad \text{(4.9)}$$

Where $Z$ was the valence number, $D$ was the diffusion coefficient, $K_B$ was the Boltzmann constant, $T$ was the absolute temperature, and $e_0$ was the fundamental electronic charge. Equation 4.10 was the modified Einstein equation, which better fit to those existing published experimental results than the original one. The $N$ in the Equation 4.10 was the number of base pairs and $m$ was a constant equal to the power law dependence of the diffusion coefficients on molecular weight [Stellwagen et al., 2003]. The results in a model relating mobility, diffusion, and different DNA sizes at room temperature.

$$\frac{\mu}{N^mD} = \frac{e_0}{K_BT} \quad \text{(4.10)}$$

$$D_{dsDNA} = 7.73 \times 10^{-6} \times N^{0.672} \text{ (cm}^2 \text{ s}^{-1}) \quad \text{(4.11)}$$

An empirical molecular diffusion equation for double strand DNA is given in Equation 4.11 [Stellwagen et al., 2003]. This was obtained by fitting published experimental results from many research groups focusing on DNA molecular diffusion in free solution. Figure 4.9 is the relationship between the diffusion coefficients and different DNA base pairs based on this empirical equation. The longer DNA had smaller diffusion coefficients.
Time was also a factor to be considered in diffusion. In the shuttle PCR, the main microchannel, from denaturation to renaturation, was 15.5 mm. So the total length of one thermal cycle was about 31 mm and twenty cycles were 620 mm. The flow rate of DNA was assumed to be 2 mm/s and the total time needed for going through 20 cycles, preheating, and postheating was 910 seconds. The diffusion length (Equation 4.12) could be calculated using Equation 4.12 [Nguyen et al., 2002], where $\tau$ was the diffusion time, $d$ was the diffusion distance, and the $D$ was the diffusion coefficient. Figure 4.10 was the resulting plot of the variation on molecular diffusion length using the empirically-derived diffusion coefficient (Equation 4.11). In Figure 4.10, the largest diffusion length was about 370 micrometers for the shortest DNA with longest time, 1000 seconds.

Figure 4.10: The diffusion length of DNA based on an empirical diffusion coefficient (Equation 4.11).
\[ \tau = \frac{d^2}{2D} \quad \text{(4.12)} \]

Equation 4.12 was derived using a short rodlike model, it had good agreement with experimental diffusion for short fragment DNA (Figure 4.11). The divergence at high molecular weight was possibly due to the coiling of DNA about itself and could be described better using a wormlike model [Eimer et al., 1991].

\[ D_t = \frac{K_B T [\ln(L_c/d) + \nu]}{3\pi\eta L_c} \quad \text{(4.12)} \]

Where \( D_t \) was the theoretical diffusion coefficient, \( K_B \) was the Boltzmann constant, \( T \) was the absolute temperature, \( d \) was the diameter, \( L_c \) was the contour length of the DNA, \( \nu \) was the correction for end effect, and \( \eta \) was the viscosity of the solvent.

The analytical model of Equation 4.12 and the empirical one underlying Equation 4.11 were plotted in a comparison in Figure 4.11. The upper plot shows the two different diffusion coefficients according to Equations 4.11 and 4.12 and the lower plot was the difference between them.

Initially the diffusion coefficient from the theoretical equation was larger than the empirical one. At roughly 620 base pairs, the two curves crossed and the empirical diffusion coefficient began to become bigger than the theoretical one. At roughly 2000 base pairs, the difference between these two curves became stable up to 5000 base pairs.

Figure 4.12 shows the diffusion length as a function of time and DNA size. Figure 4.13 was the molecular diffusion length difference between the empirical and theoretical models. The largest difference was about 14 micrometers at approximately 80 base pairs with longest time in Figure 4.13, 1000 seconds.

Figure 4.12 showed the molecular diffusion length of the DNA cocktail in the shuttle PCR at room temperature based on the theoretical model while Figure 4.13 gave the difference between the two models at room temperature.
Figure 4.11: The comparison between empirical and theoretical equations at room temperature. The upper plot has two diffusion coefficient curves in a function of different DNA sizes based on theoretical equation (Equation 4.12) and empirical equation (Equation 4.11) and the lower plot was the difference between the two equations as a function of different DNA size.

4.4.3 DNA Macromolecular Diffusion Considering Temperature

The diffusion coefficient based on empirical and theoretical models at room temperature does not reflect true PCR conditions. Temperature was also a factor in diffusions especially in a temperature-varying device like the PCR. Equation 4.12, the theoretical equation, was used to investigate the temperature impact.

Figure 4.12: Diffusion length plot based on theoretical molecular diffusion equation in a function of different size of DNA, different diffusion time, and room temperature.
Figure 4.13: The molecular diffusion length difference of empirical and theoretical equations.

Figure 4.14 shows the diffusion coefficient predicted by Equation 4.12, theoretical equation, while considering temperature effects from 0°C to 100°C. The diffusion coefficient increased significantly with temperature increase especially for short DNA segments. The predicted diffusion coefficient was an order of magnitude larger than that at room temperature. The temperature effect was relatively small for large DNAs.

Figure 4.14: The DNA macromolecular diffusion coefficient based on the theoretical model while considering temperature effects.
4.4.4 Other Diffusion Sources

There were other diffusion sources which also affected the DNA sample during electrokinetic operation. Hydrostatic unbalance moved DNA samples without applying any voltage and this unbalance could have the opposite direction. Electroosmotic flow (EOF) needed to be overcome before applying the voltage. In the shuttle PCR, EOF had the opposite direction to the desired DNA flow direction because the native polycarbonate surface had a negative charge. Hydrostatic dispersion and EOF-dominated the behavior of DNA in the microchannel.

Dongqing Li’s group had done some experiments to study the unbalanced hydrodynamic pressure induced from different reservoir levels. Their suggestion was to increase the diameter of reservoir or elongate the microchannel length. The bigger diameter the reservoirs could minimize the rise of the level of buffer and decrease the unbalanced pressure. The longer the microchannel was also to minimize the level rise effect but lost the benefit of the smaller layout. In the shuttle PCR, the diameter of the reservoir was 1.5mm and its depth was 0.75mm. The volume of each reservoir was 1.325µl and the volume of total microchannel was 0.11625 µl. The volume ratio of the reservoir volume to microchannel volume was 8.7% so it might cause different level of reservoirs after manipulating DNA cocktail toward the same direction and difficulty in control of the DNA cocktail.

4.5 Microfabrication of Shuttle PCR Chips

LIGA microfabrication was described in Section 3.2 and it was one method for fabricating the CFPCR mold insert. The fabrication technology for the shuttle PCR prototype was micromilling of the mold insert.

4.5.1 Microfabrication Process of Shuttle PCR

The design of shuttle PCR was drawn using AUTOCAD (AutoDesk, San Rafael, CA). The length of shuttle PCR chip was 21.5 mm, width was 10 mm, and the thickness was 1mm. the microchannel was 15.5 mm composed of 4 mm for denaturation, 6.5 mm for extension, 3
mm for renaturation, and two 1 mm bridge between each zone.

![Diagram of shuttle PCR prototype][1]

**Figure 4.15:** The dimensions of the shuttle PCR prototype.

<table>
<thead>
<tr>
<th>Shuttle PCR Dimension</th>
<th>Length: 21.5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Width: 10 mm</td>
</tr>
<tr>
<td></td>
<td>Thickness: 1 mm</td>
</tr>
</tbody>
</table>

| Microchannel Dimension | Total length: 15.5 mm | Denaturation | 4 mm |
|------------------------|-----------------------|--------------|
| Width: 50 µ            |                       | Renaturation | 6.5 mm |
| Depth: 150 µ           |                       | Extension   | 3 mm |

**Table 4.2:** The dimensions of electrokinetic shuttle PCR chip and microchannel.

![Microfabrication process sequence][2]

**Figure 4.16:** The microfabrication process sequence of the shuttle PCR.

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Figure 4.16 shows the microfabrication process sequence for the shuttle PCR. The first step was the micro milling of the brass mold insert described in next section. The next four steps were hot embossing, flycutting, shaping, and annealing.

![Micro Milling Machine](image)

Figure 4.17: The Kern micro milling machine in the Center for Bio-Modular Microsystems at Louisiana State University.

### 4.5.2 Micro Milling of Shuttle PCR Mold Insert

The micro milling machine, consisting of a moveable stage, tool holder, computer control, and a microscope, was used to make shuttle PCR mold insert. Figure 4.17 shows the micro milling machine (Kern MMP – Microtechnic, Murnau-Westried, Germany). The spindle was able to achieve 40,000 rpm and the machining dimension was dependent on the micro milling bit. The design drawing was converted using GIBBS CAM/CAD software (GibbsCAM 2004, Moorpark, CA) to convert an Autocad file into a patterning process.

The micro milling machine was a convenient method for mold insert fabrication because it was faster than LIGA micro fabrication. (Figure 4.18) The shuttle PCR mold insert was made by micro milling machine. The drawbacks of this method included limited aspect ratio because of the ratio of micro milling bit working length to its diameter, considering its stiffness during process; the corner radius was dependent on the diameter of the micro milling
bit and it was impossible to make a right angle corner; the wall roughness was also an issue needed to be handled. The machined surface was rough especially at the edge of the structure; burrs remained, even after using sonication to clean the mold insert. The roughness might change the dynamic behavior of the chemical reagents and exacerbated dispersion or diffusion. The minimum radius of the corner was 25 micrometers as seen in Figure 4.15.

4.5.3 Hot Embossing, Flycutting, Thermal Bonding, and Leakage Testing

The microstructure was transferred into 2 mm thick polycarbonate chips using the PHI (City of Industry, CA) hot embossing (Figure 4.16). The chips were then flycut to 0.75 mm thick left with a tolerance 5-10 micrometers from the backside using a Optimal 120 (Precitech Inc.,) flycutting machine. The surface finish using the flycutting machine was better than that of the micro milling machine and it was good enough for possible optical experimental processes.

The chips were thermally bonded with a 0.25 mm polycarbonate sheet to seal the microchannel. Before processing, the chips and glasses were cleaned using IPA, DI water, and compressed air to remove unwanted dust and particles. The temperature, time, and pressure of thermal bonding were different for different microstructures so the parameters were determined empirically. The conditions applied for the shuttle PCR were 160°C for 10 minutes while the chips were sandwiched between two clean glass plates in the oven (Watlow, Bloomington, MN). After 10 minutes, the chips were kept in the oven while the temperature was ramped down slowly to release thermal stress.

Figure 4.18: The shuttle PCR.
4.6 Prototype Shuttle PCR Experiment Preparation

4.6.1 Jig Design, Fabrication, and Assembly

A good, stable connection with the buffer was an important point for successful experiments with electrokinetic drive. A jig was designed and fabricated to offer those functions. Figure 4.19 is an exploded view of the jig, which had two separate layers. The top layer had four electrodes on it and that were already aligned with the reservoir positions of shuttle PCR chip after assembly. The bottom layer, a copper heating stage, was used to carry the shuttle PCR chip during the experiment and offered a uniform temperature distribution for each temperature zone. The top layer and the bottom layer of the jig were aligned and assembled using four screws while the four holes of the top layer were a little bigger than the screws to give some tolerance for electrode alignment within reservoirs during experiment.

Figure 4.20: The jig with shuttle PCR chip after assembly.
The jig could be repeatedly utilized with different chips; the shuttle PCR chip could be slipped into the recess of the bottom layer carried by the copper plates with thermal paste (OT-210, Omega, Stamford, CA) between them. The top layer was positioned with electrodes aligned with the reservoirs by eye and the two wing nuts were applied for fixation. The four electrodes were connected to the wires from the power supply.

4.6.2 Dynamic Coating

The EOF was described in 4.2.2 and 4.4.4 and it was an electrokinetically induced bulk fluid movement having the opposite direction to the DNA electrophoretic flow. Dynamic coating was used to generate a thin coating on the microchannel surface to reduce or reverse EOF depending on the pre-coating solution applied. The purpose of the dynamic coating was to make electroosmosis of the buffer solution and electrophoresis of the DNA sample plug have the same direction and eliminate EOF induced dispersion.

Before dynamic coating, the dispersion of the DNA plug especially at the regions close to the microchannel walls as the electrical double layer was trying to pull the buffer solution in the opposite direction of the DNA flow. Dynamic coating was realized using UltraTrol Dynamic Pre-Coatings (UltraTrol HN, TargetDiscovery, CA).

The connectors (Nano-Port F-126H, Upchurch Scientific, Oak Harbor, WA) specially designed for lab-on-a-chip applications were glued on the shuttle PCR chips to offer a reliable dynamic coating environment. One reservoir was connected to a syringe (Model 1725, Hamilton, Reno, NV) via a capillary tube (1577, Upchurch Scientific, Oak Harbor, WA) while the other reservoirs were connected to a waste bottle. The syringe was fixed on a syringe pump (Harvard 22, Harvard Apparatus, Holliston, MA) for a stable pressure-driven coating process. The first run was TBE buffer (T4073, Sigma-Aldrich) for 5 minutes to remove unwanted particles and clean the microchannel. The second run was highly-reverse precoating solution for 10 minutes and another 5 minutes of TBE buffer run followed to wash the precoating solution away. The precoating solution had to be pumped into the
microchannel continuously or it would accumulate in the microchannel and make the microchannel surface much rougher.

4.6.3 EOF (Electroosmosis Flow) Measurement Using a Microchannel Chip

In order to calculate the voltage needed for injecting the DNA sample and moving the DNA plug in the main microchannel, the EOF after dynamic coating was measured using a single microchannel chip. The steps of dynamic coating for the single microchannel chip were the same as those applied to the shuttle PCR chip.

Measurement of the EOF was done using different concentrations of TBE buffers, 1X and 10X having different electrical resistances, which gave different currents going through the microchannel. The 1X TBE was loaded into the two reservoirs then 200mV were applied for initial positioning. The current was monitored and recorded using a homemade software. When the current was at steady-state, the 10X TBE was loaded into the reservoir in which the electroosmosis flow was already flowing. The current increased sharply and achieved another steady-state while the initial 1X TBE in the microchannel was replaced totally by 10X TBE with smaller electrical resistance.

Different dynamic coating times were used to search for the best coating result. For the most highly reverse EOF, 10 minute and 25 minute coating processes were compared. The time needed for higher concentration TBE, 10X TBE, to replace the initial TBE, 1X TBE, was 75 seconds for 10 minutes coating and 78 seconds for 25 minutes coating. The mobility was calculated using Equation 4.13.

\[ \mu = \frac{L}{t \times E} \]  

Where \( \mu \) was resulted mobility, \(-1.539 \times 10^{-4}\) cm²/V·sec, \( L \) was the microchannel length, 15.5 mm, \( t \) was the time(s), 78 seconds, and \( E \) was the applied voltage, 200 mV.

4.6.4 Calculation of Operation Voltages

Electrokinetic injection was used as a tool to deliver reagents into the main microchannel. The cross configuration is a common approach and many capillary separation
chips use it. Other configurations have been developed, including double-T form or triple-T form [Ermakov et al., 2000, Lin et al., 2004, Fu et al., 2003]. Injection is an important technology in analysis because it affects the capillary separation resolution. Leaking at the intersection is the primary reason for unsuccessful injection and poor resolution [Fu et al., 2003].

A mathematical model for calculating applied voltages for different cross configurations was developed based on Kirchhoff’s law, the algebraic sum of the voltage drops for loop had to be zero, to calculate the applied voltage for each reservoir. Assumptions were equal channel width and constant conductivity. Li [Li, 2004] showed some simulation and experimental results for larger sample plugs made by using longer injection times in a cross microchannel. The drawbacks of the method were more diffusion and greater control complexity. The microchannel layout chosen for the shuttle PCR was a double-T because it allowed the sample plug length to be determined by distance the between two T intersections.

In the shuttle PCR, the distance between the two T intersections (L1 + L2 in Figure 4.21) was 2 mm and the DNA plug volume was 0.15 nl. The equations for calculating the applied voltages and values are listed in Table 4.2. An equivalent electric potential gradient was used to prevent flow in the X-direction during the loading process and the Y-direction during the dispensing and shuttling steps [Fu et al., 2002]. In Figure 4.21, the total L was 15.5 mm, L1 was 1.025 mm, L2 was 0.975 mm, and H was 4 mm. The applied voltages were calculated using parameters listed, which were physical dimensions of electrokinetic shuttle PCR.

### 4.6.5 Steady-State Temperature Performance Using Feedback Control

Transient thermal simulations, Section 4.3.3, and a lumped-parameter thermal model, Section 4.3.4, were analyzed. The time needed for achieving steady-state predicted by simulations was 350 seconds in Figure 4.7 and Figure 4.8. Feedback control was used in practice to obtain faster response, more steady-state, and overcoming environmental disturbances.
A shuttle PCR chip with buffer injected into the microchannel was inserted into the jig described in Section 4.6.1. Each heater (HK5567R5.7L12B, Minco, Minneapolis, MN) was attached to the backside of a copper block to achieve each target temperature while the thermocouples (Omega, Stamford, CA) were inserted into small grooves milled on the copper blocks.

Figure 4.21: The double-T microchannel configuration [Fu et. al, 2002]

Figure 4.22 gives the three experimental temperature response curves which had data points collected from a thermometer (HH306, Omega, Stamford, CA). The time needed for the experiment was about 250 seconds, faster than the 350 seconds predicted from simulation due to the feedback control.

Figure 4.22: The three temperature curves collected from thermometer under feedback control.
Table 4.3: Equations for calculating applied voltages for sample injection, dispensing, and control [Fu et al., 2002].

<table>
<thead>
<tr>
<th>Steps</th>
<th>Equations</th>
<th>Voltage Applied Values (Volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Φ₁</td>
<td>$\Phi_1 = \left(\Phi_3 + \Phi_4\right) \times \left(1 - \frac{L_1}{L}\right) \times L$</td>
<td>410</td>
</tr>
<tr>
<td>Φ₂</td>
<td>$\Phi_2 = \left(\Phi_3 + \Phi_4\right) \times \left(1 + \frac{L_2}{L}\right) \times L$</td>
<td>467</td>
</tr>
<tr>
<td>Φ₃</td>
<td>Ground</td>
<td>0</td>
</tr>
<tr>
<td>Φ₄</td>
<td>Initial value</td>
<td>227</td>
</tr>
<tr>
<td>Sample Dispensing and Moving Sample from 95°C to 55°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Φ₁</td>
<td>Ground</td>
<td>0</td>
</tr>
<tr>
<td>Φ₂</td>
<td>Initial value</td>
<td>586</td>
</tr>
<tr>
<td>Φ₃</td>
<td>$\Phi_3 = \left(\Phi_1 + \Phi_2\right) \times \left(1 - \frac{L_1}{L}\right) \times H$</td>
<td>71</td>
</tr>
<tr>
<td>Φ₄</td>
<td>$\Phi_4 = \left(\Phi_1 + \Phi_2\right) \times \left(1 + \frac{L_2}{L}\right) \times H$</td>
<td>123</td>
</tr>
<tr>
<td>Moving Sample from 55°C to 95°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Φ₁</td>
<td>Initial value</td>
<td>586</td>
</tr>
<tr>
<td>Φ₂</td>
<td>Ground</td>
<td>0</td>
</tr>
<tr>
<td>Φ₃</td>
<td>$\Phi_3 = \left(\Phi_1 + \Phi_2\right) \times \left(1 + \frac{L_1}{L}\right) \times H$</td>
<td>81</td>
</tr>
<tr>
<td>Φ₄</td>
<td>$\Phi_4 = \left(\Phi_1 + \Phi_2\right) \times \left(1 - \frac{L_2}{L}\right) \times H$</td>
<td>71</td>
</tr>
</tbody>
</table>

4.7 Shuttle PCR Experiment, Results, and Analysis

4.7.1 PCR Sample Preparation

A homemade jig, carrying the shuttle PCR chip and electrodes, was described in 4.6.1. The four electrodes were connected to a high voltage power supply controlled by a
homemade software.

The DNA template was a 48.5 Kb λ-DNA c1857 Sam7 (usb, Cleveland, OH) and primers were designed to make a 500-bp amplification product from the target. The forward primer was a complement to the negative strand at position 7131-7155 with 25 bp in length and its sequence was 5’-GATGAGTTCGTGTCCGTACAATTGG-3’. The reverse primer was complement to positive strand at position 7606-7630 with 25 bp in length and its sequence was 5’-GGTTATCAGAAATCCAGCCACAGCGCC-3’ (Integrated DNA Technologies, Coralville, IA). The PCR cocktail contained 10X buffer which was made of 100mM Tris-HCl (PH8.3), 15mM MgCl₂, and 0.01% w/v gelatin (Sigma-Aldrich, St. Louis, MO); nucleotide was 0.2mM (usb PN77212, Cleveland, OH), template was 5.06 ng/µl, each forward and reverse primer was 1nmol, and Taq DNA Polymerase was 5 units/µl (usb PN71160, Cleveland, OH). This DNA cocktail was tested in the commercial thermal cycler to validate the suitable composition with preheating for 1 minute at 95°C, final extension for 3 minutes at 72°C, and 20 cycles of denaturation for 30 seconds at 95°C, renaturation for 30 seconds at 95°C, and extension for 1 minute at 72°C. The total time needed for application was about one and half hours.

4.7.2 500 bp DNA Amplification Experiment and Results

Figure 4.23 shows the successful amplification results for 500 bp at velocities of 1 mm/s, 2 mm/s, and 3 mm/s. The operating times for 20 cycles of each flow rate were 620 seconds, 310 seconds, and 207 seconds, respectively. From the marker, the amplification results for all flow rates were 500 bp. The amplification intensity from 1mm/s to 3mm/s did not have a significant difference like the results presented for the LSU CFPCR limiting performance (Section 3.3). The reasons might include difficulty in controlling the DNA cocktail moving in the reverse directions repeatedly and unbalanced hydrodynamic levels in the reservoirs described in Section 4.4.4.
4.7.3 Conclusion

An alternative type of polymerase chain reactor was presented using electrokinetic drive. Successful amplification is shown in Figure 4.23 at velocities from 1mm/s to 3mm/s for 500 bp sample. In the development process, steady-state and transient temperature along the microchannel was studied to understand temperature distribution and operation time after turning on a closed loop heating system. Also the molecular diffusion as a function of different base pair lengths, temperature, and time was studied using empirical and theoretical equations.

Figure 4.23: The electrokinetic shuttle PCR amplification results for 500 bp. Two lanes for markers, lane 1 is from commercial thermocycler, lane 2 is 1mm/s, lane 3 is 2mm/s, and lane 4 is 3mm/s.
CHAPTER 5: FUTURE WORKS

5.1 Future Works

In this research, two different types of PCRs were studied. CFPCR demonstrated the capability to amplify the target DNA fragment close to the biological limiting performance and electrokinetic shuttle PCR was another possibility of micro PCR. Thermal performance of both micro PCRs were investigated using numerical simulation to understand the temperature distribution to further deliver the correct dimension for thermal management. Thermochromic liquid crystal gave ideas of non-uniformity of polycarbonate CFPCR device and motivations for understanding thermal management.

The ultimate goal of developing micro PCR is to integrate with other functional micro devices to become an automation micro system, lab-on-a-chip or micro total analysis system. The future work of this research should be optimize this micro PCR as a short goal since a efficient system depends on each efficient components and PCR is one of the critical component. Also minimizing the size of current PCR and that will increase the possibilities of a PCR array or dramatically decrease the chemical reagent demand of PCR. Developing interface mechanism including microfluid, heat transfer, and detection, between each micro component will be another interesting topic, which will bring the capability to integrate different components into a powerful system to deliver a simple output from a complicated raw sample.
REFERENCES


Liu, J., Enzelberger, M., Quake, S., “A nanoliter rotary device for polymerase chain reaction”,


Stellwagen, N. C., Gelfi, C., Righetti, P. G., “The free solution mobility of DNA”,

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## APPENDIX: COMPARISON AMONG DIFFERENT PCRS

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sample Volume</th>
<th>Reaction time</th>
<th>Material</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng, et al. 1996</td>
<td>12 µl</td>
<td>30 min</td>
<td>Silicon-glass</td>
<td>30</td>
</tr>
<tr>
<td>Daniel, et al. 1998</td>
<td>2 µl</td>
<td>8 min 30 sec</td>
<td>Silicon</td>
<td>30</td>
</tr>
<tr>
<td>Giordano, et al, 2001</td>
<td>1.7 µl</td>
<td>4 min</td>
<td>polyimide</td>
<td>15</td>
</tr>
<tr>
<td>Shoffner, et al. 1996</td>
<td>28 µl</td>
<td>30 min</td>
<td>Silicon-glass</td>
<td>35</td>
</tr>
<tr>
<td>Woolley, et al.1996</td>
<td>50 µl</td>
<td>26 min 15sec</td>
<td>Si-glass</td>
<td>35</td>
</tr>
<tr>
<td>Kalinia, et al. 1997</td>
<td>10 nl</td>
<td>30min</td>
<td>Quartz glass</td>
<td>40</td>
</tr>
<tr>
<td>Landers, et al. 1998</td>
<td>5-15 µl</td>
<td>14min</td>
<td>Glass</td>
<td>30</td>
</tr>
<tr>
<td>Kopp, et al. 1998</td>
<td>5-20 µl</td>
<td>50min</td>
<td>Glass-copper</td>
<td>30</td>
</tr>
<tr>
<td>Friedman, et al 1998</td>
<td>5 µl</td>
<td>20 min</td>
<td>Glass</td>
<td>35</td>
</tr>
<tr>
<td>Waters, et al. 1998</td>
<td>12 µl</td>
<td>216 min</td>
<td>Glass</td>
<td>24</td>
</tr>
<tr>
<td>Ferrance, et al. 2000</td>
<td>10 µl</td>
<td>7 min 44sec</td>
<td>Silicon</td>
<td>30</td>
</tr>
<tr>
<td>Lagally, et al. 2000</td>
<td>10 µl</td>
<td>15 min</td>
<td>Glass</td>
<td>20</td>
</tr>
<tr>
<td>Khandurina, et al. 2000</td>
<td>7 µl</td>
<td>20 min</td>
<td>Cr-Glass</td>
<td>10</td>
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<tr>
<td>Giordano, et al. 2000</td>
<td>5 µl</td>
<td>4 min 50 sec</td>
<td>Polyimide</td>
<td>15</td>
</tr>
<tr>
<td>Nagai, et al. 2001</td>
<td>1.3 pl -32 µl</td>
<td>120 min</td>
<td>Silicon</td>
<td>40</td>
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<td>Obeid, et al, 2003</td>
<td>Depending on cycles</td>
<td>Depending on cycles</td>
<td>glass</td>
<td>20-40</td>
</tr>
<tr>
<td>Study</td>
<td>Volume</td>
<td>Time</td>
<td>Material</td>
<td>Temperature</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------</td>
<td>-------</td>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>Quake, et al, 2001</td>
<td>2 µl</td>
<td>60 min</td>
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<td>40 min</td>
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<td>Shin, et al, 2003</td>
<td>2 µl</td>
<td>43 min</td>
<td>PDMS</td>
<td>30</td>
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</table>
VITA

Pin-Chuan Chen was born on March 17, 1978, in Taipei, Taiwan, to Hsien-Chin Chen and Ling-Ling Liao. He attended National Cheng-Kung University, Tainan, Taiwan, for his undergraduate education. He graduated with a bachelor of science degree, in mechanical engineering in May of 2000.

In 2002, he left for the United States where he entered the Mechanical Engineering Department at Louisiana State University in Baton Rouge, Louisiana, to pursue graduate studies. He joined the Microsystems Engineering Team (µSET) within the Mechanical Engineering Department and began working on Micro-PCR project after first semester. He expects to receive the degree of Master of Science in Mechanical Engineering in May, 2006.